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Facteurs de risque modifiables associés à l'incidence, l'élimination et la prévalence d'infections intra-mammaires chez la vache laitière en lactation

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Facteurs de risque modifiables associés à l'incidence, l'élimination et la prévalence d'infections intra-mammaires chez la vache laitière en lactation

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Résumé

La mammite subclinique est un problème de santé fréquent et coûteux. Les infections intra-mammaires (**IIM**) sont souvent détectées à l'aide de mesures du comptage des cellules somatiques (**CCS**). La culture bactériologique du lait est cependant requise afin d'identifier le pathogène en cause. À cause de cette difficulté, pratiquement toutes les recherches sur la mammite subclinique ont été centrées sur la prévalence d'IIM et les facteurs de risque pour l'incidence ou l'élimination des IIM sont peu connus. L'objectif principal de cette thèse était d'identifier les facteurs de risque modifiables associés à l'incidence, l'élimination et la prévalence d'IIM d'importance dans les troupeaux laitiers Canadiens.

En premier lieu, une revue systématique de la littérature sur les associations entre pratiques utilisées à la ferme et CCS a été réalisée. Les pratiques de gestion constamment associées au CCS ont été identifiées et différentiées de celles faisant l'objet de rapports anecdotiques.

Par la suite, un questionnaire bilingue a été développé, validé, et utilisé afin de mesurer les pratiques de gestion d'un échantillon de 90 troupeaux laitiers canadiens. Afin de valider l'outil, des mesures de répétabilité et de validité des items composant le questionnaire ont été analysées et une évaluation de l'équivalence des versions anglaise et française a été réalisée. Ces analyses ont permis d'identifier des items problématiques qui ont du être recatégorisés, lorsque possible, ou exclus des analyses subséquentes pour assurer une certaine qualité des données. La plupart des troupeaux étudiés utilisaient déjà la désinfection post-traite des trayons et le traitement universel des vaches au tarissement, mais beaucoup des pratiques recommandées n'étaient que peu utilisées.

Ensuite, les facteurs de risque modifiables associés à l'incidence, à l'élimination et à la prévalence d'IIM à *Staphylococcus aureus* ont été investigués de manière longitudinale sur les 90 troupeaux sélectionnés. L'incidence d'IIM semblait être un déterminant plus important de la prévalence d'IIM du troupeau comparativement à l'élimination des IIM. Le

port de gants durant la traite, la désinfection pré-traite des trayons, de même qu'une condition adéquate des bouts de trayons démontraient des associations désirables avec les différentes mesures d'IIM. Ces résultats viennent souligner l'importance des procédures de traite pour l'obtention d'une réduction à long-terme de la prévalence d'IIM.

Finalement, les facteurs de risque modifiables associés à l'incidence, à l'élimination et à la prévalence d'IIM à staphylocoques coagulase-négatif (**SCN**) ont été étudiés de manière similaire. Cependant, afin de prendre en considération les limitations de la culture bactériologique du lait pour l'identification des IIM causées par ce groupe de pathogènes, une approche Bayesienne à l'aide de modèles de variable à classe latente a été utilisée. Les estimés non-ajusté de l'incidence, de l'élimination, de la prévalence et des associations avec les expositions apparaissaient tous considérablement biaisés par les imperfections de la procédure diagnostique. Ce biais était en général vers la valeur nulle. Encore une fois, l'incidence d'IIM était le principal déterminant de la prévalence d'IIM des troupeaux. Les litières de sable et de produits du bois, de même que l'accès au pâturage étaient associés à une incidence et une prévalence plus basse de SCN.

Mots-clés : Mammite, vaches laitières, Canada, pratiques de gestion, comptage des cellules somatiques, *Staphylococcus aureus*, staphylocoques coagulase-négatif, questionnaire, validation, traduction.

Abstract

Subclinical mastitis is a very frequent and costly health issue that can be detected using somatic cell count (**SCC**) measurements, but requires the use of milk bacteriological culture for identification of the causal pathogen. Because of this latter difficulty, nearly all subclinical mastitis research has focused on prevalent intramammary infections (**IMI**) and less is known on risk factors for IMI incidence or elimination. The main objective of this thesis was to identify the manageable risk factors associated with the incidence, elimination, and prevalence of IMI of importance in Canadian dairy herds.

First, a systematic review of the literature on the associations between management practices used on farms and SCC was carried out. Management practices consistently associated with SCC were identified and differentiated from other practices for which anecdotic reports were available.

Then, a bilingual questionnaire was developed, validated, and employed to measure the practices used on a sample of 90 Canadian dairy herds. To validate this tool, measures of repeatability and of validity of the questionnaire's items were analyzed and an evaluation of the equivalence of the English and French versions was conducted. These analyses indicated that the questionnaire was, in general, acceptable, but also pinpointed some problematic items. These items were recategorized when possible or otherwise excluded from subsequent analyses to ensure good data quality. Most of the herds studied were already using post-milking teat disinfection and blanket dry cow therapy. Many other frequently recommended practices were not widely adopted.

Next, manageable risk factors associated with *Staphylococcus aureus* IMI incidence, elimination, and prevalence were investigated on the 90 selected herds in a longitudinal fashion. The *S. aureus* IMI incidence appeared to be a stronger determinant of the herd prevalence than the elimination rate. Among other practices, wearing gloves during milking, using pre-milking teat disinfection, and having an adequate teat end condition showed desirable associations with the outcomes. These results highlight the

importance of good milking practices to achieve a long-term reduction of *S. aureus* IMI prevalence.

Finally, manageable risk factors associated with coagulase-negative staphylococci (**CNS**) IMI incidence, elimination, and prevalence were investigated in a similar manner. Because of the greater limitations of milk bacteriological culture to identify IMI caused by this group of pathogens, a Bayesian latent class model approach was used. Sensitivity and specificity estimates from an internal validation study were used to link the observed IMI milk culture result to the latent true quarter IMI status. Non-adjusted estimates of IMI incidence, elimination, prevalence, and of associations with expositions appeared to be considerably biased by the diagnostic procedure's imperfections. Most often, estimates were biased toward the null value. Again, IMI incidence was the main determinant of the herd IMI prevalence. Sand and wood products bedding were associated with lower CNS incidence and prevalence. Sending cows to pasture was also associated with lower CNS IMI incidence and prevalence.

Keywords : Mastitis, dairy cows, Canada, management practices, somatic cell count, *Staphylococcus aureus*, coagulase-negative staphylococci, questionnaire, validation, translation.

Table des matières

Liste des tableaux

Liste des figures

Liste des sigles et des abréviations

h: hour (heure)

d: day (jour)

mo: month (mois)

yr: year (année)

IIM (IMI) : infection intra-mammaire (intramammary infection)

CCS (SCC) : comptage des cellules somatiques (somatic cell count)

SCS: somatic cell score (score des cellules somatiques)

BMSCC: bulk milk somatic cell count (comptage des cellules somatiques du réservoir de lait)

HSCC: herd average of individual somatic cell count (moyenne du comptage des cellules somatiques individuel des vaches d'un troupeau)

CMT: California mastitis test

SCN (CNS) : staphylocoques coagulase-négatif (coagulase-negative staphylococci)

S. aureus : *Staphylococcus aureus*

S. agalactiae: *Streptococcus agalactiae*

AMS : automated milking system (système de traite robotisé)

PMTD : post-milking teat disinfection (désinfection post-traite des trayons)

DCT : blanket dry cow treatment (traitement universel des vaches au tarissement)

DHI : dairy herd improvement program (programme d'amélioration des troupeaux latiers)

NCDF : National Cohort of Dairy Farms (Cohorte National de Fermes Laitières)

CBMRN : Canadian Bovine Mastitis Research Network (Réseau Canadien de Recherche sur la Mammite Bovine)

PEI : Prince Edward island (Ile-du-Prince-Edward)

MeSH: Medical subject heading

ºC: degré Celsius (degree Celsius)

µl: microlitre

ml: millilitre

kg: kilogramme (kilogram)

Se: sensibilité (sensitivity)

Sp: spécificité (specificity)

OR: odds ratio (rapports de cotes)

IR: incidence ratio (rapports d'incidence)

CI : confidence interval (intervalle de confiance)

SE: standard error (erreur type)

SD : standard deviation (déviation standard)

CCC: coefficient de corrélation de concordance (concordance correlation coefficient)

PABAK : prevalence-adjusted bias-adjusted Kappa (Kappa ajusté pour la prévalence et le biais)

MCMC : Markov chain Monte Carlo

"There are three kinds of epidemiologist:
those who can count and those who can't."
Anonymous

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Introduction

La mammite est une des maladies les plus coûteuses pour l'industrie laitière dans les pays industrialisés. En fait, ce problème de santé est constitué de deux composantes relativement distinctes et indépendantes; la mammite clinique et subclinique (Barkema et al., 1998a, Olde Riekerink et al., 2008a). Historiquement, les recherches sur la mammite ont d'abord été dirigées vers la mammite clinique, un problème de santé facilement observable et pouvant entraîner des pertes de revenus tangibles aux yeux des producteurs laitiers. Cependant, les infections intra-mammaires (**IIM**) subcliniques sont maintenant considérées par les producteurs, intervenants et chercheurs comme un problème de santé aussi sinon plus important. En effet, malgré le fait que la vaste majorité des IIM ne soit pas accompagnée de signes cliniques, celles-ci peuvent tout de même entraîner une fibrose des tissus mammaires résultant en une baisse passagère ou permanente de la production lactée. Les quartiers infectés peuvent également être source d'infection pour les quartiers sains et produisent un lait de qualité inférieure avec un comptage des cellules somatiques (**CCS**) augmenté, un potentiel de transformation fromagère réduit et une durée de conservation moindre (Klei et al., 1998; Ma et al., 2000). Les IIM qui ne sont pas accompagnées de signes cliniques sont d'ailleurs souvent diagnostiquées à l'aide du suivi du CCS individuel effectué mensuellement sur la plupart des fermes laitières. Un CCS de 200,000 cellules/ml de lait est fréquemment utilisé comme valeur seuil au-dessus de laquelle un quartier ou une vache sera reconnu comme étant infecté (Schukken et al., 2003). Plus souvent qu'autrement, cependant, on voudra identifier les pathogènes responsables des IIM dans un troupeau afin de pouvoir mieux cibler les pratiques de gestion à corriger. En effet, les différents pathogènes en cause lors d'IIM ne partagent pas nécessairement les mêmes déterminants épidémiologiques et les mesures préventives à instaurer devront donc être spécifiques à chacun de ceux-ci. La culture bactériologique du lait est alors l'outil le plus souvent utilisé afin d'identifier les pathogènes en cause. Cette méthode diagnostique présente cependant certaines limitations dont une combinaison de sensibilité (Se) et spécificité (Sp) plus ou moins satisfaisante pour certains groupes de pathogènes.

Pratiques de gestion :

De nombreuses pratiques de gestion ont déjà été associées, dans la littérature scientifique, aux IIM. Certaines pratiques de gestion recommandables ont d'ailleurs été sélectionnées depuis plusieurs décennies et constituent maintenant les différents programmes de contrôle de la mammite courrament utilisés par les vétérinaires, intervenants et producteurs laitiers (Dodd et al., 1970; Anonymous 2000. NMC recommended mastitis control program). Cependant, la vaste majorité des études utilisées pour sélectionner ces pratiques de gestion étaient des études transversales associant la prévalence d'IIM à d'hypothétiques facteurs de risque. Ce type d'étude est difficilement interprétable et peut parfois entraîner l'identification d'associations fallacieuses puisque l'ordre chronologique entre exposition et maladie n'est pas connu. De plus, étant donné les coûts associés à l'identification des pathogènes en cause, la plupart des études réalisées se contentaient d'évaluer les associations entre expositions et CCS, une mesure générale de la santé de la glande mammaire (Barkema et al., 1998b; Barnouin et al., 2004, Wenz et al., 2007). Seulement quelques études associant les pratiques de gestion à la prévalence d'IIM par des pathogènes spécifiques ont été réalisées récemment (Sampimon et al., 2009b; Piepers et al., 2011). Jusqu'à présent, une seule étude (Zadok et al., 2001) a évalué les facteurs de risque associés à l'incidence d'IIM de pathogènes spécifiques. Les facteurs de risque évalués dans cette étude, cependant, étaient strictement des caractéristiques des vaches ou des quartiers plutôt que des pratiques de gestions modifiables pouvant être modulée afin de contrôler l'incidence de ces IIM. De plus, puisque les résultats de CCS sont, en général, facilement disponibles, il n'est pas rare de trouver des études rapportant une association entre une pratique de gestion et le CCS alors qu'aucun lien biologique théorique ne peut être établi entre cette pratique et la santé de la glande mammaire. De part la quantité et la qualité de la littérature disponible, il est donc présentement difficile pour les médecins vétérinaires praticiens d'établir, en se basant sur des principes de médecine factuelle, les pratiques de gestions recommandables qui devraient être mise de l'avant dans les programmes de contrôle de la mammite couramment utilisés sur le terrain.

Questionnaires :

Dans la vaste majorité des études réalisées jusqu'à présent sur les associations entre pratiques de gestion et santé de la glande mammaire, un questionnaire « maison » était dévelopé pour les besoins de l'étude et utilisé afin de mesurer les pratiques de gestion utilisées à la ferme. Comme tout autre test diagnostique, les questionnaires possèdent une certaine précision ou répétabilité et une certaine validité. De nombreuses recommandations ont d'ailleurs été émises pour le développement et la validation des questionnaires épidémiologiques vétérinaires (DelGreco et al., 1987b; Schukken et al., 1989; Vaillancourt et al., 1991; Slater, 1997). Malgré ces recommandations, jusqu'à maintenant, aucune étude sur la santé de la glande mammaire ayant utilisé un questionnaire n'a rapporté avoir validé leur instrument de collecte de données. De plus, aucun des questionnaires utilisés n'a été publié ou rendu disponible par les auteurs pour consultation. Dans tout les cas, la qualité des données n'est pas évaluée et les analyses sont réalisées en assumant que les expositions sont parfaitement mesurées. Ce genre de pratique ne semble pas être limité à la recherche sur la santé de la glande mammaire et est fréquemment observé dans plusieurs domaines de recherche en santé animale. En effet, bien que certaines excellentes études traitant de la validation d'un questionnaire épidémiologique animal aient été publiées (Slater et al., 1992; Reeves et al., 1996; Nespeca et al., 1997; Sallander et al., 2001), plus souvent qu'autrement la validation du questionnaire utilisé est simplement laissée de côté. Cette culture scientifique est, malheureusement, bien supportée par beaucoup de revues scientifiques traitant de santé animale et qui accepte ces publications. En santé humaine, en comparaison, de nombreux questionnaires épidémiologiques sur des sujets variés ont été validés et peuvent être utilisés gratuitement par les chercheurs de différents domaines de recherche (voir http://dceg.cancer.gov/tools/design/jobmodules ou www.cdc.gov/nchs/nhanes.htm pour des exemples).

Dans plusieurs situations, le dévelopment d'un questionnaire épidémiologique bilingue ou la traduction dans une deuxième langue d'un questionnaire existant sera nécessaire (Carlson 2000; Markaki et al., 2007; Olde Riekerink et al., 2008a). Ce sera le

cas, par exemple, pour le dévelopement d'un questionnaire épidémiologique ayant comme cible une population de producteurs laitiers canadiens; où l'anglais et le français sont courrament parlés. Dans ce genre de situation, l'utilisation d'une procédure de traduction standardisée, réalisée par des experts du domaine de recherche capables de bien saisir le sens de l'outil de recherche est généralement recommandée (Brislin, 1970). L'utilisation d'une telle procédure, cependant, sert simplement à garantir une certaine constance de la traduction et n'est pas un gage d'équivalence des différentes versions de l'instrument. Une évaluation formelle de l'équivalence des différentes versions de l'instrument devrait donc être réalisée de manière indépendante. Présentement, il existe peu de recommandations sur l'évaluation de l'équivalence des versions d'un questionnaire épidémiologique multilingue. En effet, la littérature disponible actuellement sur le sujet et développée principalement dans les domaines de la recherche interculturelle et de l'éducation s'adresse essentiellement aux tests psychométriques fréquemment utilisés dans ces domaines (Sireci, 1997; Carlson, 2000). Quoique les questionnaires épidémiologiques et ces tests psychométriques puissent présenter des similarités, ils présentent également des différences importantes. Les tests psychométriques permettent en général d'attribuer un score à un individu en fonction des réponses de celui-ci à de multiples questions relativement liées entre elles. Ce score est par la suite utilisé comme une mesure générale d'un concept relativement précis. L'équivalence inter-culturelle et inter-langage des tests psychométriques est fréquemment évaluée à l'aide de méthodes où différents groupes d'examinés souvent unilingues, parfois bilingues, répondront chacun à une seule des versions du test. Certains items du test seront utilisés comme point d'ancrage auquel le score global sera comparé (Sireci, 1997). Cette approche est clairement inadéquate dans le cas d'un questionnaire épidémiologique constitué d'éléments relativement indépendants qui feront l'objet d'analyses indépendantes. Dans ce cas, l'équivalence inter-langage de chacun des éléments constituant le questionnaire devra être démontrée.

Pathogènes de la mammite d'importance au Canada:

Dans une large étude terrain sur des troupeaux du nord-est des États-Unis (Schukken et al., 2009), une très large proportion du CCS du réservoir de lait des troupeaux ayant un CCS élevé (CCS réservoir > 200,000 cellules/ml de lait) pouvait être attribué aux IIM à *Staphylococcus aureus* ou à *Streptococcus agalactiae*. Dans une étude récente réalisée sur un échantillon aléatoire de troupeaux laitiers canadiens, cependant, une proportion très faible (<1%) d'échantillons de réservoir de lait étaient positif pour *S. agalactiae*, suggérant la quasi-éradication de cet agent pathogène au Canada (Olde Riekerink et al., 2010). Dans cette même étude, par contre, la prévalence de troupeau ayant au moins une vache infectée par *S. aureus* était estimée à 74%. Ces résultats sont corroborés par ceux de Reyher et al. (2011). Dans cette dernière étude, *S. aureus* était retrouvé dans 2 à 3% des échantillons de lait provenant des quartiers de vaches en lactation au Canada; ce qui en faisait le deuxième pathogène de la mammite le plus fréquemment rencontré. L'effet des IIM à *S. aureus* est considérable; dans une méta-analyse réalisée par Djabri et al. (2002) les quartiers infectés présentaient un CCS géométrique moyen de 333,000 cellules/ml de lait (95% CI : 320,000 – 348,000) comparativement à 68,000 cellules/ml de lait pour les quartiers non-infectés. Les pertes économiques associées à une IIM subclinique à *S. aureus* étaient estimées en 1997 à 170$ USD par lactation (Wilson et al., 1997). De plus, les IIM à *S. aureus* sont, en général, capable de persister dans la glande mammaire, peuvent être transmisent aux autres vaches du troupeau durant la traite et ont la capacité de résister aux traitements conventionnels (Barkema et al., 2006). Les IIM à *S. aureus* demeurent donc un problème d'importance dans les troupeaux laitiers canadiens.

Dans l'étude de Reyher et al. (2011), le groupe de pathogènes le plus fréquemment retrouvé chez les vaches en lactation au Canada était les staphylocoques à coagulase négative (SCN), avec une prévalence de 5 à 7% d'échantillons positifs. Dans plusieurs études, réalisées dans différents pays, les SCN constituaient la cause la plus fréquente d'IIM et ce groupe de pathogènes est maintenant considéré comme pathogène émergent (Tenhagen et al., 2006; Pyörälä and Taponen, 2009; Sampimon et al., 2009a). Même si la

plupart de ces IIM ne sont que temporaires et n'entraîneront qu'une modeste élévation du CCS, la fréquence élevée d'IIM par ces pathogènes peut se solder par une augmentation réelle du CCS du réservoir de lait. Les résultats de Schukken et al. (2009) semblent d'ailleurs indiquer que, dans les troupeaux à bas CCS (< 200,000 cellulles/ml), les SCN seraient responsables de la plus grande proportion des problèmes de santé de la glande mammaire. Ces IIM pourraient donc être un obstacle important à l'amélioration de la santé de la glande mammaire au Canada, en particulier dans les troupeaux à bas CCS.

Biais de mauvaise classification :

Peu d'efforts ont été faits jusqu'à présent en recherche sur la santé de la glande mammaire pour réconcilier les résultats de culture bactériologique du lait avec le statut d'IIM réel des quartiers. D'ailleurs, ce fait n'est pas unique à la recherche sur la mammite bovine et peut être constaté dans pratiquement tous les domaines de recherche épidémiologique, des côtés animal et humain. Dans beaucoup de publications scientifiques, des efforts importants semblent être alloués au dévelopement de modèles statistiques complexes et précis alors que les biais dûs à la qualité des données utilisées dans ces modèles sont simplement occultés ou ignorés. Malgré l'existence de plusieurs méthodes quantitatives permettant de mesurer ou de corriger l'effet des biais de mauvaise classification telles, entre autres, les méthodes Bayesiennes (McInturff et al., 2004), les méthodes pour données manquantes (Greenland, 2005) et la simulation Monte Carlo (Lash et al. 2009), l'effet de ces biais est généralement discuté de manière strictement qualitative. Hors, l'évaluation qualitative des biais se résume à un exercice de raisonnement dans un contexte d'incertitude; un exercice auquel le cerveau humain échoue, en général, de manière prévisible (Lash, 2007). Dans ces évaluations qualitatives, l'erreur systématique et l'incertitude sont, à toutes fins pratiques, invariablement sous-estimées (Lash, 2007).

Cette problématique s'avère particulièrement importante lorsque la combinaison de sensibilité (**Se**) et de spécificité (**Sp**) du test diagnostique utilisé pour identifier le statut

infectieux des individus étudiés est relativement imparfaite et entrainera des biais plus ou moins important tout dépendamment de la fréquence de la maladie dans la population. Ces biais ne seront pas limités aux estimés de fréquence des maladies, mais affecteront aussi les mesures d'association entre exposition et maladie, de même que les intervalles de confiance autour de ces estimés (Tarafder et al., 2011). Dans le cas de l'erreur de classification non-différentielle d'une variable discrète, le biais dans la mesure d'association avec une exposition hypothétique sera, le plus souvent, vers la valeur nulle. Dans bien des cas, cependant, l'absence de contrôle de ce biais ménera à des erreurs de type I et II, donc à des conclusions erronées (Gustafson et Greenland, 2006; McGlothlin et al., 2008; Tarafder et al., 2011). Sous certaines conditions, l'ampleur du biais de mauvaise classification sera extrêmement limitée, et un ajustement pour ce biais serait alors, à toute fins pratiques, superflu. Ce sera le cas, lorsqu'un événnement est relativement rare (< 5%) et que le test utilisé pour son diagnostique possède une Sp élevée (> 95%). Ce sera également le cas pour une maladie très fréquente (> 95%) combinée à un test diagnostique démontrant une excellente Se (> 95%).

Concrètement, pour les IIM causées par des pathogènes tels *S. aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, ou *Klebsiella* spp, qui ont une faible prévalence et une faible incidence et pour lesquels la spécificité de la culture bactériologique du lait est excellente (Dohoo et al., 2011; Elmoslemany, communication personnelle), l'ampleur du biais de mauvaise classification est certainement extrêmement limitée et ne justifie probablement pas l'utilisation de méthodes quantitatives d'analyse de biais. Pour des pathogènes plus fréquents, cependant, tels les SCN, les corynebacterium et les entérocoques, l'ampleur du biais serait certainement suffisante pour justifier un tel ajustement.

Souvent, lors d'une étude longitudinale de type cohorte, le même test diagnostique sera initialement utilisé afin de constituer une cohorte d'individus non-infectés qui pourront par la suite est suivi dans le temps. Dans ces cas, les Se et Sp imparfaites du test diagnostique pourront mener à l'inclusion ou à l'exclusion innapropriée de certains

individus et donc à un biais de sélection. Dans le cas d'une étude cohorte sur l'incidence d'IIM, par exemple, on voudra s'assurer que les individus recrutés sont tous bel et bien à risque de contracter la maladie (tous initialement non-infectés). Pour arriver à cette fin, une méthode permettant de maximiser la Se du test diagnostique pourrait être utilisée. Dans le cas des SCN, l'utilisation d'une définition d'IIM à SCN basée sur la culture de $\geq$ 100 cfu de SCN/ml de lait, plutôt que de $\geq$ 200 cfu de SCN/ml (Dohoo et al., 2011) serait avantageuse dans le contrôle de ce biais de sélection pour ce pathogène. Aussi, l'utilisation, en début de cohorte, d'échantillons pairés interprétés en parallèle serait une autre manière intéressante de contrôler ce biais de sélection (Dohoo et al., 2012).

Finalement, l'impact de la mauvaise classification des expositions est également bien connu (Gustafson, 2004; Höfler, 2005) et peut, dans certaines situations, entraîner des biais importants et parfois imprévisibles dans les estimés d'association et de leur erreur type. De même, des méthodes quantitatives d'ajustement ont été développées (Gustafson, 2004; Lash et al., 2009) afin de contrôler ces biais. Ces biais peuvent cependant aussi être simplement contrôlés en s'assurant de la qualité de l'outil de collecte de données utilisé.

Objectifs

Ce projet visait à étudier l'épidémiologie des IIM durant la période lactée dans les troupeaux laitiers canadiens. L'objectif était d'identifier les facteurs de risque modifiables associés à l'acquisition, l'élimination et la prévalence d'IIM par certains agents pathogènes d'importance. Les objectifs spécifiques étaient :

1. Distinguer les pratiques de gestion ayant démontrées dans la littérature une association constante avec le CCS, une mesure générale de la santé de la glande mammaire, de celles faisant l'objet de rapports anecdotiques;

2. Développer et valider un outil bilingue permettant de mesurer les pratiques de gestion utilisées sur les fermes laitières canadiennes;

3. Identifier les pratiques de gestion associées à l'incidence, l'élimination et la prévalence d'IIM à *Staphylococcus aureus* durant la période lactée tout en prenant en considération les autres pratiques et conditions en place à la ferme;

4. Identifier les pratiques de gestion associées à l'incidence, l'élimination et la prévalence d'IIM à SCN durant la période lactée tout en prenant en considération les autres pratiques et conditions en place à la ferme ainsi que les difficultés diagnostiques liées à l'identification de ces IIM.

5. Évaluer l'impact des limitations de la culture bactériologique du lait pour le diagnostique des IIM à SCN sur les mesures de fréquence de ces IIM et d'association avec les facteurs de risque.

Invited Review: Impact of udder health management practices on herd somatic cell count

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Abstract

A systematic review of the scientific literature on relationships between management practices used on dairy farms and herd SCC was undertaken to distinguish those management practices that have been consistently shown to be associated with herd SCC from those lacking evidence of association. Relevant literature was identified using a combination of database searches (PubMed, Medline, CAB, Agricola, and Web of Science) and iterative screening of references. To be included in the review, a manuscript had to be published after 1979 in French, English, or Dutch; study design had to be other than case report or case series; herds studied had to be composedof ≥ 40 milking cows producing on average ≥7,000kg of milk in 305 d; interventions studied had to be management practices applied at the herd level and used as udder health control strategies; and SCC had to be measured using electronic cell counting methods. The 36 manuscripts selected were mainly observational cross-sectional studies; 8 manuscripts dealt exclusively with automatic milking systems and 4 with management of calves and heifers and its effect on SCC in early lactation heifers. Most practices having consistent associations with SCC were related to milking procedures: wearing gloves during milking, using automatic take-offs, using post-milking teat dipping, milking problem cows last, yearly inspection of the milking system, and use of a technique to keep cows standing following milking; all were consistently associated with lower herd SCC. Other practices associated with lower SCC were the use of a free-stall system, sand bedding, cleaning the calving pen after each calving, surveillance of dry-cow udders for mastitis, use of blanket dry-cow therapy, parenteral selenium supplementation, udder hair management, and frequent use of the CMT. Regarding SCC of heifers, most of the consistent associations reported were related to interventions made during the peri-partum period. Studies on automatic milking systems have frequently reported elevation of the herd SCC following transition to the new system. These elevations seemed to be mediated both by the lack of monitoring of chronically infected cows and by an elevated incidence of intramammary infections. By assembling the results reported in many different studies, this review generates a more comprehensive

understanding of the management practices influencing SCC and highlights areas of SCC control knowledge that lack evidence of effectiveness.

Key words: management, somatic cell count, systematic review, automatic milking system

Introduction

There is a vast body of literature reporting associations between various management practices applied on dairy farms and different measures of udder health. Some of the udder health parameters that have been most frequently studied are the incidence of clinical mastitis, SCC, and, to a lesser extent, the pathogen-specific prevalence and incidence of IMI. As a measure of udder health, SCC is a very interesting and valuable measure. SCC is mainly determined by IMI; it is therefore an excellent proxy to measure prevalence and even incidence of IMI whether clinical signs of mastitis are present or not (Dohoo and Leslie, 1991).

In addition, SCC measurements can easily be obtained for research either from bulk milk (**BMSCC**) or as a herd average of individual cow (**HSCC**) measurements from DHI programs.

Finally and most importantly, BMSCC, along with total bacterial plate count, is used internationally as a standard for milk quality. For dairy producers worldwide, SCC is not only a measure of herd udder health performance; it is also a determinant of the marketability of their milk. In a recent Canadian study, 88% of dairy producers claimed that they usually review the individual SCC data of their herd on the very same day the report was available (Dufour et al., 2010). Dairy producers are indeed very concerned with SCC and will frequently inquire about the management practices that could best help them achieve BMSCC reductions.

For dairy veterinary practitioners and extension agents, providing evidence-based advice to clients is a difficult task. The number of studies reporting associations between

management practices and SCC is vast. In addition, due to the relative availability of SCC measurements, it is not uncommon to find studies reporting associations between SCC and management practices that are not even intended for use as udder health control strategies. Finally, in many studies, interventions are evaluated at the cow level without providing evidence for herd-level effects. These studies do not take into account the within-herd dynamics of IMI. Although these studies do provide important knowledge and understanding of mastitis epidemiology, the results observed when an intervention was applied to a few individuals within a herd can differ from the ones that would have been observed if the intervention had been applied to the whole herd.

The objective of this study was to perform a standardized review of the literature on associations between management practices used on dairy farms and herd-level SCC. A specific objective was to distinguish between management practices that have consistently shown association with SCC when applied at the herd level, and management practices for which evidence of an association with herd-level SCC is lacking.

Materials and Methods

Literature Search and Inclusion Criteria

Five databases, PubMed, Medline, CAB, Agricola, and Web of Science were searched on April 22, 2009 for original research published in French, English, or Dutch. To ensure that the retrieved manuscripts would be relevant for modern dairy herds, searches were restricted to manuscripts published later than 1979. Search strategies were developed with the help of a librarian and consisted of Boolean search statements using Medical subject heading (**MeSH**) terms specific to each database. MeSH is a system of medical metadata consisting of sets of terms naming descriptors in a hierarchical structure that permits searching at various levels of specificity. The MeSH terms used were descriptors of the population (dairy cows) and outcome (SCC) of interest. The Boolean search

strategies and MeSH terms used were “cattle” and “cell count” and “milk” for Medline and PubMed, “dairy cattle” and “somatic cell count” for CAB, “cow” and “somatic cell count” for Agricola, and “cow” or “cows” or “cattle” or “bovine” and “somatic cell count” for Web of Science. Manuscripts retrieved from the different databases were collated and duplicates were eliminated. Only manuscripts for which an abstract was available were considered.

To be included in the review, a manuscript had to meet the additional following criteria:

1. Intervention studied was a management practice applied or observed at the herd level and used as an udder health control strategy;
2. SCC was measured using cell counting methods rather than California Mastitis Test (**CMT**) or Rapid Mastitis Test (Immucell, Portland, ME);
3. Study design was not case report or case series;
4. Mean 305-day milk production of the herds studied was ≥ 7,000 kg; and
5. Mean herd size of the herds studied was ≥ 40 milking cows.

Thresholds for these two last inclusion criteria were defined based on the 25th percentiles for herds participating in dairy herd improvement programs in Canada in 2008 (Sylvia Lafontaine, Valacta, Sainte-Anne-de-Bellevue, Quebec, personal communication). These thresholds were defined to select studies realized with dairy herds comparable to modern dairy herds found in North America and in most European countries.

A search protocol was developed based on the recommendations of Greenhalgh and Peacock (2005), who identified electronic search and reference tracking as the most powerful methods for identifying high quality sources. In short: all abstracts obtained were reviewed concurrently by two of the authors (S.D and A.F) using the previously defined inclusion criteria. All reviewers were blinded to the authors, journal, and year of publication of the manuscripts. At this stage of the reviewing process, inclusion criteria were not strictly applied in order to not exclude any relevant manuscript. Whenever, the

two reviewers disagreed on the selection of an abstract, a third author (D.T.S.) was asked to review the abstract and decide on its eligibility for inclusion. Full texts of the selected abstracts were then obtained, reviewed, and selected in a similar manner with strict application of the inclusion criteria. Whenever information relative to inclusion criteria were lacking in a manuscript, companion papers were consulted or efforts were made to contact authors by electronic communication to obtain the missing information. Finally, the list of references quoted in each paper was screened to find potentially relevant manuscripts that had not been identified by the databases searches. Additional manuscripts found were reviewed as described previously, and their reference lists were screened until complete depletion.

Data Abstraction

Two of the authors (S.D. and A.F.) concurrently abstracted the following information from the selected manuscripts on a standardized form: study design, study location, study period, number of herds, explanatory variables studied, and specific outcome variables studied. For studies with multiple published papers, the most complete paper was used as the primary source of information and the other reports were used for supplemental information. For each manuscript, one of the authors (S.D.) abstracted or computed effect estimates for each management practice for which results were reported. Only one effect estimate per study per comparison was computed for a given management practice. Whenever a study reported more than one effect estimate or more than one measure of association with SCC for a specific practice, priority was given to herd effect estimates rather than to group-specific effect estimates, then to incidence data rather than to prevalence data, then to continuous measures of SCC or somatic cell score (**SCS**) rather than to proportion of cows over a given SCC or SCS threshold, and finally to multivariable analyses rather than to unadjusted analyses or to descriptive results. Whenever more than one multivariable model was presented, the most complete model was used.

Standard error for each effect estimate was abstracted, computed, or imputed using variance imputation methods (Follman et al., 1992; Philbrook et al., 2007; O'Rourke and Greenland, 2008) and 95% confidence intervals (**CI**) were computed using the obtained effect estimate and SE. These 95% CI were adjusted using the Bonferroni correction (Abdi, 2007) whenever multiple comparisons were made.

Analytical Methods

Associations between management practices and SCC were first investigated using forest plots, a graphical representation summarizing the individual effects that were observed in the selected studies (Khan et al., 2003). In view of the large amount of heterogeneity among SCC measurements used, definitions of explanatory variables, and measures of association reported, no attempt was made to compute pooled effect estimates. Hence, formal analyses were restricted to non-parametric comparisons of study results. Each of the reported associations between a management practice and SCC was classified as being toward lower SCC, a null effect, or toward higher SCC. For those studies employing categorical-scale measurement of herd SCC or of herd-proportion of high SCC cows, this was done by noting if the odds ratio (**OR**) of belonging to the lower SCC category when using the investigated practice was greater than , equal to, or lower than the null value of 1.0, respectively. For studies investigating the incidence of SCC increases, this was done by noting if the incidence ratio (**IR**) comparing incidences in herd using and not using the investigated practice was lower than, equal to, or greater than the null value of 1.0, respectively. Finally, for studies investigating differences of herd SCC or of herd-proportion of high SCC cows between herds using and herds not using a practice, this was done by noting if the difference was lower than, equal to, or greater than the null value of 0.0, respectively. A reported effect was considered globally statistically significant whenever its corrected 95% CI did not include the null effect.

Management practice effect estimates that were reported in multiple articles were tested for consistency of the association with SCC by a binomial test; consistency being

defined as continually having an association in a given direction. This binomial test estimates the probability of observing a given proportion of associations in one direction, under the null hypothesis of no association (unidirectional sign test; O'Rourke and Greenland, 2008). We must emphasize that this binomial test does not have high statistical power, especially when very few reports of effect estimates are available. We therefore used a more liberal *P*-value of 0.15 as the threshold for statistical significance of the binomial test.

Results

A flowchart describing the selection process of the relevant literature is presented in Fig. 1. During the full text reviewing process, important information related to our inclusion criteria were missing for 22 articles. For two articles, missing information could be found in companion papers. Authors of the remaining 20 manuscripts were contacted electronically and asked to provide the needed information; answers were obtained for 14 manuscripts and 8 were included in the review. For six of the manuscripts with missing information (Vecht et al., 1989; Hogeveen et al., 2001; Gygax and Nosal, 2006; Köster et al., 2006a; Köster et al., 2006b; Stup et al., 2006), we were unable to obtain a reply from the authors even after multiple attempts to contact them; these manuscripts were not included in the review.

The main features of the 36 manuscripts included in this study can be found in Table I. All studies included in this systematic review were observational studies; the vast majority used a cross-sectional study design (n=28) or a combination of cross-sectional and before-and-after designs (n=6). One study used strictly a before-and-after study design (Rasmussen et al., 2001) and only one study used a longitudinal cohort design (Bareille et al., 1998).

Heterogeneity in exposure measurements was noticeable. For 25 manuscripts, questionnaires were used to measure a part or the totality of the management practices of interest; only two independent studies have used the exact same questionnaire (Svensson et al., 2006; Nyman et al., 2009). For the remaining studies, the questionnaires were developed specifically for the project. Only one study reported validating, although only partly, the questionnaire used (Barnouin et al., 2004).

In many manuscripts, more than one SCC-related outcome was investigated. There was substantial heterogeneity among outcomes used between manuscripts (see Table I). Briefly, three different outcomes were reported:

1. Herd or group-specific SCC or SCS (estimated from bulk milk (n=14) or from mean individual cow SCC (n=16)),
2. Prevalence of cows with a SCC over a specific threshold (n=6), and
3. Proportion of cows with a SCC increasing from under to above a specific threshold over a defined period of time (n=2).

Herd or group specific SCC or SCS were frequently (n=12) categorized in two (low and high) to three (low, medium, and high) categories. Definitions of each category were specific to each study, with low SCC herd definitions ranging from less than 150,000 cells/ml to less than 264,000 cells/ml, and high SCC herd definitions ranging from more than 200,000 cells/ml to more than 700,000 cells/ml. In four manuscripts (Bareille et al., 2000; De Vliegher et al., 2004; Svensson et al., 2006; Nyman et al., 2009), a measure of SCC in heifers at the beginning of their first lactation was the outcome studied. In these studies, practices investigated were related to calf and heifer management from birth up to a few weeks following calving.

Results for management practices with effect estimates reported by two or more different studies are summarized in Table II. For many of the practices investigated, effect estimates were reported only once. Furthermore, many practices were sometimes measured in such a specific manner in one study that reporting the results obtained with those of other

studies would be misleading. These results are therefore presented individually. For these distinct practices, a list of the practices with statistically significant associations is presented in Table III. Other single-reported practices for which non-significant associations were obtained are presented as supplementary material (Table IV).

In addition to the results reported in Table III, one study realized by Lievaart et al. (2007) used data collected by Barkema et al. (1998b) to specifically investigate associations between management practices and variation of HSCC within low (< 150,000 cells/ml), medium (150-200,000 cells/ml), and high (> 200,000 cells/ml) SCC categories. In complement to the findings of Barkema et al. (1998b), they observed that within the low SCC category, herds using a wet pre-milking teat preparation had higher HSCC. They also found conflicting significant associations from one season to another for herds using a technique to keep cows standing after milking. During winter, for herds in the low SCC category, feeding cows or feeding and locking cows in headlocks following milking was associated with higher HSCC; during summer, a reverse association was observed with herds in that same low SCC category having lower HSCC when using these techniques. In this same study, the number of days before milk was added to the bulk tank following calving was negatively associated with HSCC within the low SCC category. Opposite associations were also observed in this study for feeding fresh milk to calves: within the low SCC category, this practice was associated with higher HSCC; while within the high SCC category, it was associated with lower HSCC. Finally, similarly to Barkema et al. (1998b) findings, clipping udder hair of all cows every year was associated with lower HSCC for herds within the medium and high SCC categories.

In two instances, divergent significant associations were reported within a study (not reported in Table III). Mattresses in dry-cow cubicles were associated with greater odds of being a medium SCC herd than a low or high SCC herd (Barkema et al., 1998b). Cows were moved to the calving pen earlier before calving in medium SCC herds than in low or high SCC herds (Barkema et al., 1998b).

Automatic Milking Systems

Eight studies specifically investigated the effect of milking with an automatic milking system (**AMS**) (Klungel et al., 2000; Van der Vorst and Hogeveen, 2000; Rasmussen et al., 2001; Billon and Tournaire, 2002; Rasmussen et al., 2002; Van der Vorst et al., 2002; Van der Vorst et al., 2003; Van der Vorst and Ouweltjes, 2003). In these studies, contrasts were made between periods before and after installation of the AMS, between herds milking with an AMS and a conventional milking system, and between years of installation of the AMS. For studies realized within the same country, data from herds used in earlier studies were most often reused in more recent studies in addition to data from newer AMS users. Results will be presented only for the most recent and complete datasets.

In 262 Dutch and 99 Danish AMS herds, geometric mean BMSCC in the period following AMS installation (period ranged from the first 6 to first 18 months following installation) was significantly higher than before installation (period ranged from the preceding 6 months to preceding 4 years before installation); for all generations of AMS, Dutch herds had geometric mean BMSCC after AMS installation of 204,000 cells/ml compared to 170,000 cells/ml before and Danish herds 279,000 cells/ml after installation compared to 259,000 cells/ml before (Van der Vorst et al., 2002). No significant difference in BMSCC before and after introduction of AMS were seen for 33 German herds with geometric mean BMSCC before of 201,000 cells/ml and after of 203,000 cells/ml (Van der Vorst et al., 2002). The German AMS producers who agreed to participate in this study, however, were a relatively small proportion (25%) of all German herds using an AMS at that time compared to 90% for Danish AMS herds and 80% for Dutch AMS herds. In a similar study on 46 (23%) of the 200 French dairies equipped with AMS, no significant difference in BMSCC was found between AMS and conventional milking herds (Billon and Tournaire, 2002). In this study, herds using AMS had a mean BMSCC of 230,000 cells/ml before installation compared to 244,000 cells/ml after installation.

Similar changes of BMSCC over time following AMS installation were seen for all generations of AMS in Denmark, Germany, and The Netherlands: an increase BMSCC was observed just after introduction of the AMS followed by a slow decrease over time, with AMS herds reaching a BMSCC level comparable to conventional milking herds from within 1 yr to 1 yr and a half of installation (Van der Vorst et al., 2002). In the Danish and German AMS herds, no association between year of installation of the AMS and change in BMSCC over time during the period following installation could be seen. In the 262 Dutch herds, however, AMS installed between January 1, 1998 and March 31, 1999 (defined as 2nd generation AMS) had BMSCC level comparable to conventional milking herds in the first 6 mo and in the 12- to 18-mo periods following installation. The Dutch herds using other generations of AMS, however, had a significantly higher BMSCC than conventional herds for up to a 1 yr and a half following installation.

In a study by Rasmussen et al. (2001) conducted earlier on 69 Danish AMS herds, the BMSCC rise after installation of the AMS could be explained, in part, by a significantly increased incidence of cows going from under to over 200,000 cells/ml. In this study, cumulative incidence went from 10% in the year before installation to 15% in the year after installation, leading to a prevalence of cows over 200,000 cells/ml of 35% before to 39% after installation ($P < 0.01$). In Denmark, dairy producers changing from conventional to AMS milking were offered participation in a self-monitoring program to help them accomplish the transition between systems. This self-monitoring program relied mostly on detection followed by treatment or culling of clinically infected and high SCC cows both before and during the milking system transition (Rasmussen et al., 2002). Herds enrolled on this self-monitoring program, like herds not enrolled, had significantly higher BMSCC in the year following installation of the AMS compared to the year before installation; however, they had a significantly lower increase in BMSCC than herds not enrolled (Rasmussen et al., 2002). In these Danish AMS herds, no significant influence of the monitoring program on the incidence of new high SCC cows could be highlighted (Rasmussen et al., 2001).

In a study by van der Vorst and Ouweltjes (2003), BMSCC in the year preceding the transition to an AMS was identified as an important predictor of the BMSCC in the year following installation (38% of variation explained) and of change in BMSCC after the transition (10% of variation explained). In this same study, dairy producers waiting for a longer time before replacing milk liners and waiting for a system indication to change them had respectively a higher BMSCC and a larger BMSCC increase in the year following transition to an AMS. On the other hand, BMSCC was significantly lower on AMS farms where teats were cleaned more than once (15 s each time). In addition, herds where cleaning of the AMS was set automatically and where udders were shaved at least twice a year had smaller increase in BMSCC in the year following transition to the AMS. Finally, when adjusting for the adaptation period by excluding the first 6 months following the transition to AMS, herds cleaning the floor of the waiting area with an automatic scrapper and herds using additional mechanical ventilation had significantly lower BMSCC (Van der Vorst et al., 2003).

Discussion

Methodological Strengths and Limitations

The most valuable outcome of this systematic review was perhaps the ability to identify practices that have shown consistent associations with SCC in different populations, under different circumstances, and across time. Demonstrating consistency of an association is not related in any way to significance testing methods and, in this regard, the analytical methods used in this review were very appropriate for this purpose (Rothman et al., 2008). Demonstrating consistency of an association is a clear step toward the identification of cause and effect. The practices that have shown a consistent association with SCC possibly had a larger impact on SCC, were efficient under different production

settings, or were efficient against most commonly encountered mastitis pathogens. For these reasons, they should be part of our initial recommendations to any dairy producer.

Lack of consistency, on the other hand, cannot be used to rule out a cause and effect association. Numerous studies did not report any effect estimate for management practices that yielded what the authors considered to be a non-significant association. Many of the practices discussed in this review therefore had their associations with SCC completely reported in few, often only one, manuscripts. The binomial test used is certainly biased to some extent by these unreported results. In addition, the different treatments of confounding effects that were used in different studies could have lead to the reporting of different directions of association, thus leading to an apparent inconsistency of the results reported. This apparent inconsistency would be caused by the presence of residual confounding of the observed associations in some studies and not in others, rather than being caused by an ineffectiveness of a specific management practice. With that many studies being conducted over such a large period of time in many different countries, we can expect that several different confounders are somehow operating. The practices that have not shown consistent associations with SCC should therefore not be completely ruled out. Further research would, however, be advocated on these practices or on their conditions of application.

The manuscripts selected in this systematic review represent an important piece of the published literature on associations between management practices applied on dairy farms and SCC. Our specific average herd size and milk production inclusion criteria, however, did restrict the studies selected and careful readers should, therefore, restrict the application of these results to similar dairy herds. The decision to select only studies for which the interventions were applied to or observation were made on the entire herd was driven by the need to evaluate evidences for management practices that have been tested in a manner similar to how they would be applied as interventions. Studies excluded because of interventions being applied to a few individuals within a herd do provide ample knowledge on mastitis but clearly cannot fully demonstrate the generalized herd-level

applicability of an intervention. Some potentially important manuscripts were excluded for purely logistical reasons: linguistic limitations and failure to obtain important information from authors. Linguistic limitations are a common feature of most published systematic reviews (Moher et al., 2007). In our study, considering the retention percentage of the English, French, and Dutch abstracts (1.1%), the probability of excluding an important manuscript due to the linguistic restrictions was very low (0.2%). The resulting potential linguistic bias was therefore, in our opinion, very limited. Failure to obtain important missing information from authors is potentially a more important problem. The 6 manuscripts excluded for this reason had otherwise good potential for being included in this review. It might be argued, however, that incomplete reporting of information highlighted them as poorer quality studies. Although these studies could not be included, they are nonetheless listed to inform readers of additional potentially useful information.

A major limitation of this review was the need to focus on direction of associations rather than on the magnitude of associations. This limitation resulted mainly from the lack of comparability between studies. The analysis approach used was for this reason very rudimentary; it would classify OR of 0.30 and 0.90 both as "associated with lower SCC" and weight equally all studies regardless of sample size and standard error (**SE**). On the other hand, transformation of the results from the different studies on a common scale would have required an extensive amount of supplemental information and, with many of the retained studies having been published more than a decade ago, this alternative was simply not feasible.

All of the studies selected were observational studies and nearly all used cross-sectional or before-and-after study designs which typically do not provide the highest level of evidence. Observational studies are often hypothesis-generating and the causality of the associations highlighted by such studies should be further explored using experimental designs. In our review no experimental studies conducted at the herd level could be found. For many of the practices discussed, however, results have been published from experimental studies conducted at the cow level with animals or sometimes quarters within

a herd being randomized to control and treatment groups. Also, for some practices, experimental studies at the herd or cow level have been realized but with udder health outcomes other than SCC. To better understand the significance of the associations reported in this review, we recommend evaluating published results from such experimental studies in addition to the consistency of association reported.

Although experimental studies conducted at the herd level are rather uncommon, we would have nevertheless expected to find a certain number of observational studies using sounder study designs such as cohort and case-control designs. A major problem encountered with cross-sectional study designs is the impossibility to record time order of occurrence between exposure and outcome leading to the risk of wrongfully highlighting reverse associations between management practices and SCC. As an example, producers having a herd with a high SCC might be keener to include fore-stripping in their milking procedures in order to detect clinical mastitis cases. A cross-sectional study design would only highlight the association between fore-stripping and higher SCC, an association that is correct but can certainly not be interpreted as causal.

Management Practices and SCC

Relatively few of the numerous management practices investigated demonstrated consistent associations with SCC. Furthermore, many of the practices frequently recommended in mastitis control programs had a quite limited amount of published information available on their effectiveness in a conventional dairy setting; with many showing even inconsistent directions of association with SCC across studies. One must bear in mind though, that many of the practices proposed in mastitis control programs are intended primarily to prevent clinical mastitis rather than a high HSCC. Independence between these two udder health components has been highlighted before (Barkema et al., 1998a; Olde Riekerink et al., 2008) and practices affecting one or the other component could therefore differ.

In relation to milking procedures, the body of literature supports that milkers should definitely wear gloves during milking, use (well adjusted) automatic milking unit take-offs, and apply post-milking teat disinfection (**PMTD**). Furthermore, high SCC cows and clinical mastitis cases should be milked last. Using a specific milking unit for these cows or rinsing, cleaning, or disinfecting the unit after these cows are milked and before first lactation cows are milked, were studied only once and were associated with higher SCC. The milking parlor should be kept clean and good performance of the milking system should be ensured by having the system inspected at least annually. Although keeping cows standing after milking seemed associated with lower SCC in many studies, the findings of Lievaart et al. (2007) highlighted some of the conditions (seasons) potentially limiting the general utility of this practice. In their study, no biological explanation of the observed season effect on this practice could be proposed; they did however recognize, like others (Barkema et al., 1998b; Barnouin et al., 2004; De Vliegher et al., 2004), the effect of herd manager attitudes on herd SCC. Associations between dairy producers' attitudes, management practices used, and udder health have been reported before (Barkema et al., 1999; Jansen et al., 2009). It is probable that many of the effect estimates reported by the selected studies are strongly confounded by herd managers' attitudes; associations observed with lower SCC may in fact be the result of having a knowledgeable and motivated herd manager, which may in turn be associated with both the use of a technique to keep cows standing following milking and lower SCC (through other management techniques). Results from a recent study measuring the association between individual cow post-milking standing time and IMI incidence, thus not confounded by herd manager attitude, seemed to confirm this hypothesis (DeVries et al., 2010). In this study increasing post-milking standing time was in fact associated with a higher IMI incidence. In another study measuring the association between incidence of IMI by coagulase-negative staphylococci and delivery of feed around milking, a strategy commonly used to promote longer standing time following milking, again an increased IMI incidence was observed for herds using the practice (Dufour et al., 2008).

Few housing related interventions yielded very consistent associations with SCC. Based on the combined results of several studies, a sound recommendation is to use a free-stall housing system with sand bedded cubicles. Such a modification of the housing system, however, is clearly not a minor correction. A needed modernization of the existing facilities on a farm, however, should be seen as an opportunity to recommend this type of housing system. Cleanliness or frequency of cleaning of the calving pen was the only other management practice related to housing that could be consistently associated with SCC.

From our results, the most well supported recommendation remains to administer an approved intramammary antibiotic treatment to all cows at dry-off. Selective antibiotic treatment at dry-off is not consistently associated with lower SCC. The criteria used to select cows to be treated were not discussed in studies of selective dry-off treatment. The efficiency of a selective treatment is certainly determined by the correct selection of the cows to be treated and therefore specification of the selection criteria would have been crucial. One must bear in mind, though, the dual objectives of a dry-cow antibiotic treatment: prevention of new IMI during the dry period and cure of existing IMI (Dingwell et al., 2003). While it might be relatively easy to select cows to be treated in order to achieve the second objective, it will always be difficult, if not impossible, to accurately select and treat the cows that would have otherwise acquired a new IMI. The first objective of a dry-cow antibiotic treatment can therefore hardly be fulfilled by a selective dry-cow treatment alone and the merit of a selective dry-cow treatment is probably heavily determined by the pathogen-specific dry period IMI incidence rate of the herds using it.

Daily inspection of dry cows' udders to detect mastitis during the dry period was also consistently associated with lower SCC. Such an inspection however, if not coupled with a specific and practical intervention, would not lead to reduced herd SCC. Similarly, use of the CMT was continuously associated with lower SCC. No details concerning any interventions associated with these practices could be found in the studies reporting these associations and it is possible that the associations observed were again an indirect measure of the effect of a superior dairy producer attitude toward controlling mastitis. Also,

frequent clipping or flaming of udder hairs and parenteral supplementation with selenium were both consistently associated with lower herd SCC.

The attitude of the dairy producer toward culling needs to be modified to achieve lower SCC. In order to reduce their SCC, dairy producers need to have proactive and well defined culling strategies based on udder conformation, teat lesions, and clinical mastitis cases rather than simply reacting to udder health events with the result being a higher number of cows culled for mastitis and high SCC (Bareille et al., 1998; Barkema et al., 1998b; Barkema et al., 1998a; Barnouin et al., 2004; Rodrigues et al., 2005).

In many studies, an attempt to measure associations between specific components of the diet and SCC was made and very conflicting results were obtained. In these studies, the effects on SCC of the different components of the ration were always estimated independently, without taking into account the interaction of a component with the other elements of the diet. It is obvious that the effect of feeding a specific type of feed, mineral, or supplement is absolutely determined by the presence or absence of other components of a diet. Furthermore, this effect is also dependant on the current herd status for the nutrient in question; feeding additional amount of concentrates will usually be beneficial to energy-deprived cows but could be harmful to well fed cows. As another example, vitamin E can have either an oxidant or anti-oxidant effect depending on the cow initial vitamin E status (Bouwstra et al., 2010). Therefore, studying the association of SCC with any individual component of the diet in such a manner is not very instructive.

Similarly, many studies have reported associations between different components of the milking system and SCC. Again, inconsistent results were observed and they might be the result of the inappropriate partition into its measurable constituents of a system that can only be correctly assessed as a whole.

Only a few relatively recent studies investigated calf and heifer management as risk factors for elevated SCC. It is well accepted that an important proportion of heifers already have an IMI at the moment of their first calving (Oliver and Mitchell, 1983; Pankey et al.,

1991; Fox et al., 1995). Although many of these IMI may be of short duration, the resulting elevated SCC observed early in the lactation is often associated with elevated SCC throughout the whole lactation (De Vliegher et al., 2004; Paradis et al., 2010). Because a rather high proportion of herds is usually composed of first lactation cows, the impact of these IMI on the herd SCC cannot be ignored. It is interesting to observe that, although practices used from birth to first calving were investigated, most of the practices significantly associated with heifers' early lactation SCC were interventions used during the few weeks before and around calving time. This relatively short period of time is potentially of great importance for acquisition of new IMI and further research to understand the risks and mechanisms of IMI and host response in this specific period should be undertaken.

Automatic Milking System

The introduction of AMS in dairy production is relatively recent and there is a growing interest in this new technology. Although the use of an AMS cannot directly be considered as an udder health control strategy, AMS can nonetheless have an impact on the choice and condition of application of milking procedures, on mastitis monitoring, and, therefore, on SCC. An augmentation of the BMSCC following installation of an AMS has not been observed in every country but it has been reported often, and dairy producers having an already elevated BMSCC before installation of the AMS seemed more at risk of experiencing such an augmentation and to a greater magnitude (Billon and Tournaire, 2002; Van der Vorst et al., 2002; Van der Vorst and Ouweltjes, 2003). To prevent or reduce such an adverse effect, dairy producers should focus their efforts on reducing their herd SCC before an AMS is put into place. To achieve this objective they could, similarly to the recommendations made in the Danish self monitoring program, intensify the detection and culling of problematic cows beforehand (Rasmussen et al., 2001; Rasmussen et al., 2002). One of the major difficulties encountered with AMS is to correctly sort milk from high SCC cows and clinical mastitis cases; the treatment or culling of some of these cows before

installation of the AMS is therefore a sound recommendation. In one study, however, a part of the increase in herd SCC could be explained by an increase number of new high SCC cows (Rasmussen et al., 2001). The increase in SCC seemed therefore not to be explained solely by the milking of problematic cows but also by an increase number of new IMI following implementation of the AMS technology. Practices used once the AMS is in place should therefore also be addressed. At this time, little is known on practices associated with SCC in AMS herds; dairy producers that were more aggressive regarding maintenance and cleaning of the AMS seemed to be able to mitigate the SCC elevation. Efficiency of the AMS teat cleaning methods seems to be another key point that could limit a SCC augmentation (Van der Vorst et al., 2003; Van der Vorst and Ouweltjes, 2003). Future research on AMS should be aimed at identifying risk factors associated with the incidence of IMI, rather than those associated with IMI prevalence.

Conclusions

A large number of management practices have shown consistent associations with herd-level SCC when used in usual dairy settings. These practices should be the cornerstone of udder health recommendations to dairy producers. Although many management practices have shown interesting associations with SCC, the lack of consistency observed should moderate reliance on their use.

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Tables

Table I. Characteristics of manuscripts on associations between management practices and measures of somatic cell count

Manuscript and index number	Country	Study period	Population	Study design [a]	Outcome	Intervention or observation
1. Bach et al., 2008	Spain	2006	47 herds	CS	Herd mean BMSCC	Multiple [b]
2. Bareille et al., 1998	France	1995-1996	237 herds	LC	Incidence of cows increasing to >200,000 cells/ml	Multiple [b]
3. Bareille et al., 2000	France	1995-1997	228 herds	CS	Herd % of heifers with SCC >200,000 cells/ml between 7-45 DIM	Heifer management
4. Barkema et al., 1998a[1]	Netherlands	1992-1994	274 herds	CS	Herd geometric mean BMSCC: LOW (<150,000 cells/ml, n=85) MED (151-250,000 cells/ml, n=133) HI (>250,000 cells/ml, n=56)	Culling rate
5. Barkema et al., 1998b[1]	Netherlands	1992-1994	201 herds	CS	Same as Barkema 1998a	Multiple [b]
6. Barnouin et al., 2004	France	1999-2001	534 herds	CS	Herd mean SCS: LOW (percentiles 0-5 of SCS, n=326) MED (percentiles 50-55 of SCS, n=208)	Multiple [b]
7. Bartlett et al., 1992	USA (OH)	1988-1989	48 herds	CS	Log_n of herd mean BMSCC	Multiple [b]
8. Bewley et al., 2001	USA (WI)	1999	244 herds	CS	Herd mean SCS	Barn design, bedding, and cooling method
9. Billon and Tournaire, 2002	France	2001	101 herds	BA, CS	Herd mean BMSCC	Use of AMS
10. De Vliegher et al., 2004	Belgium	1999-2000	159 herds	CS	Herd geometric mean SCC of heifers between 5-14 DIM	Management of heifers

Table I. (Continued)

Manuscript and index number	Country	Study period	Population	Study design [a]	Outcome	Intervention or observation
11. Ellis et al., 2007	UK	2003-2004	28 herds	CS	Herd geometric mean BMSCC	Cows cleanliness
12. Erskine et al., 1987a[2]	USA (PA)	---	32 herds	CS	Herd mean SCC: LOW (≤150,000 cells/ml, n=16) HI (≥700,000 cells/ml, n=16)	Multiple [b]
13. Erskine et al., 1987b[2]	USA (PA)	---	32 herds	CS	Same as Erskine et al., 1987a	Se, vitamine A, E, and β-carotene
14. Erskine and Eberhart, 1991	USA (PA)	---	71 herds	CS	Herd mean SCC: LOW (≤250,000 cells/ml, n=24) HI (>700,000 cells/ml and high IMI prevalence, n=47)	Post-milking teat disinfection and use of dry-cow therapy
15. Fulwider et al., 2007	USA (WI, MN, NY, IA, IN)	2005-2006	113 herds	CS	Herd mean SCC	Stall length, width, bedding, and cow cleanliness
16. Goodger et al., 1988	USA (CA)	1984-1985	50 herds	CS	Herd mean SCC: LOW (<264,000 cells/ml, n=25) HI (>264,000 cells/ml, n=25)	Multiple [b]
17. Hutton et al., 1990[3]	USA (WA)	1986-1987	59 herds	CS	% of cows with SCS ≤4: LOW (28 herds with the highest %) HI (31 herds with the lowest %)	Multiple [b]

Table I. (Continued)

Manuscript and index number	Country	Study period	Population	Study design [a]	Outcome	Intervention or observation
18. Hutton et al., 1991[3]	USA (WA)	1986-1987	59 herds	CS	Same as Hutton et al., 1990	Multiple [b]
19. Jayarao et al., 2004	USA (PA)	2000-2001	126 herds	CS	Herd geometric mean BMSCC	Multiple [b]
20. Khaitsa et al., 2000	USA (OH)	1996-1997	186 herds	CS	Herd mean BMSCC	Multiple [b]
21. Klungel et al., 2000[4]	Netherlands	1996-1998	105 herds	BA, CS	Log_n of herd mean BMSCC	Use of AMS and milking frequency
22. Lievaart et al., 2007[1]	Netherlands	1992-1994	246 herds	CS	Herd monthly SCC (within 3 categories) LOW (<150,000 cells/ml, n=81) MED (150-200,000 cells/ml, n=86) HI (>200,000 cells/ml, n=79)	Multiple [b]
23. Nyman et al., 2009	Sweden	2005-2006	72 herds	CS	Herd % of heifers with SCC ≥200,000 cells/ml at first test Log_n of heifers SCC at first test	Management of heifers
24. Rasmussen et al., 2001[5]	Denmark	1997-2000	69 herds	BA	Herd mean SCC % of cows increasing >200,000 cells/ml % of cows with SCC >200,000 cells/ml	Use of AMS
25. Rasmussen et al., 2002[5]	Denmark	1997-2001	98 herds	BA, CS	Log_{10} of herd mean BMSCC	Use of AMS and self monitoring program

Table I. (Continued)

Manuscript and index number	Country	Study period	Population	Study design [a]	Outcome	Intervention or observation
26. Rodrigues et al., 2005	USA (WI)	2001-2004	180 herds	CS	Herd mean log_{10} BMSCC and SCS Herd mean SCS % of cows with SCS >4 Herd mean BMSCC (categorical): LOW (<250,000 cells/ml, n=36) MED (250-400,000 cells/ml, n=83) HI (>400,000 cells/ml, n=61)	Multiple [b]
27. Smith and Ely, 1997	USA (GA)	1994	178 herds	CS	Herd mean SCC	Multiple [b]
28. Smith et al., 2002	USA (39 states)	1998-2000	10,754 herds	CS	Herd mean SCC and SCS % of cows with SCS 0-3 and 7-9	Milking frequency
29. Svensson et al., 2006	Sweden	1998-2000	102 herds	CS	Heifer SCC ≥200,000 cells/ml at first test	Heifer management
30. Van der Vorst and Hogeveen, 2000[4]	Netherlands	1997-1999	167 herds	BA, CS	Log_n of herd mean BMSCC	Use of AMS and milking frequency
31. Van der Vorst et al., 2002[4,5]	Denmark, Germany, and Netherlands	1997-2001	729 herds	BA, CS	Log_n of herd mean BMSCC	Use of AMS and milking frequency
32. Van der Vorst et al., 2003[4]	Netherlands	1997-2001	114 herds	CS, BA	Herd mean BMSCC (categorized)	Use of AMS, housing, milking, and others

Table I. (Continued)

Manuscript and index number	Country	Study period	Population	Study design [a]	Outcome	Intervention or observation
33. Van der Vorst and Ouweltjes, 2003[4]	Netherlands	1997-2002	28 herds using AMS	CS	Log_n of herd mean BMSCC: LOW (<170,000 cells/ml, n=5) MED (170-265,000 cells/ml, n=14) HI (>265,000 cells/ml, n=9)	Housing, milking, and others in AMS herds
34. Weiss et al., 1990	USA (OH)	---	9 herds	CS	Herd mean BMSCC	Vitamin E and Se
35. Wenz et al., 2007	USA (21 states)	2002	1,013 herds	CS	Producer-reported mean BMSCC: LOW (<200,000 cells/ml, n=264) MED (200-400,000 cells/ml, n=569) HI (>400,000 cells/ml, n=180)	Multiple [b]
36. Wilson et al., 1995	USA (NY,PA)	1992-1994	76 herds	CS	Herd mean BMSCC	Segregation of *Staph. aureus* infected *cows*

[a] CS: Cross-sectional study, BA: Before-and-after study LC: Longitudinal cohort study.

[b] Study reporting a wide range of management practices.

Studies sharing the same superscript were conducted using data from some of the same herds during the same period.

Mean is the arithmetic mean unless specified otherwise.

BMSCC: Bulk milk SCC.

SCC: Individual cow SCC (in cells/ml) as measured by DHI organizations.

Table II. Summary of the results of direction of associations estimated between dairy farm management practices and measures of somatic cell count that were reported by more than one study.

Practice used	Low SCC		High SCC		Study index number [1]	*P*-value [2]
	n [3]	Sig [4]	n [3]	Sig [4]		
Milking procedures						
Milk 3 times/day	5	4	3	2	26, 28, 30, 35	0.22
Wear gloves*	5	2	0	---	1, 18, 26	0.03
Fore-strip	2	0	4	1	19, 26, 35	0.23
Pre-milking teat disinfection	3	0	1	0	17, 26	0.25
Dry teats	3	1	1	0	5, 12	0.25
Individual drying towels	3	0	2	0	7, 12, 17, 26	0.31
Post-milking teat disinfection (PMTD)[5,]*	8	6	0	---	2, 5, 12,14, 18, 20	<0.01
PMTD during winter	1	0	2	1	35	0.38
Sprayed PMTD (*vs* dipped)	2	1	3	1	5, 6, 19	0.31
Automatic take-off*	8	5	4	2	5, 17, 19, 26, 27, 35	0.12
High SCC and/or clinical mastitis cows milked last[5,]*	3	3	0	---	6, 18, 36	0.13
Milking system inspected ≥ once/year*	6	1	2	0	5, 12, 17, 26	0.11
Milking parlor cleaned regularly[5,]*	4	0	0	---	5, 18	0.06
Cows locked after milking*	4	2	0	---	5, 6	0.06
Housing						
Housed in free-stall[5,]*	6	5	1	0	7, 20, 27, 35	0.06
Housed primarily on pasture[5]	2	2	3	1	2, 7, 35	0.31
Water or humidity in pasture[5]	0	---	2	1	6, 17	0.25
Increased stocking density[5]	1	1	1	0	2, 17	0.50
Sand bedding*	5	2	0	---	8, 19, 35	0.03
Mattress in cubicles[5]	4	3	1	0	15, 23, 35	0.16
Decreased bedding moisture %[5]	3	1	5	0	7, 18, 35	0.22
Cleaner cows[5]	2	1	0	---	7, 11	0.25
Slatted floor in alleys	5	2	2	0	5, 10, 35	0.16
Alleys are flushed	1	0	3	3	16, 35	0.25

Table II. (Continued)

Practice used	Low SCC		High SCC		Study index number [1]	*P*-value [2]
	n [3]	Sig [4]	n [3]	Sig [4]		
Housing (continued)						
Cows calved in calving pen	3	3	1	0	3, 6, 7, 18	0.25
Calving pen cleaned after each calving[5,]*	4	3	0	---	5, 6	0.06
Cows and heifers left in calving pen < 1 d after calving	2	1	0	---	10, 23	0.25
Dry period						
Udder checked for mastitis daily*	4	2	0	---	2, 5	0.06
Dry-cows' housing regularly disinfected[5]	2	1	2	0	5, 6	0.38
Blanket dry-cow treatment (*vs* selective or none)*	11	4	1	0	5, 12, 14, 18, 26, 35	<0.01
Selective dry-cow treatment (*vs* none)	3	0	1	0	12, 35	0.25
Others						
Use PMTD and blanket dry-cow treatment	2	2	0	---	12, 14	0.25
Selenium supplementation of milking cows	2	2	0	---	13, 34	0.25
Parenteral selenium supplementation*	4	3	0	---	13, 35	0.06
Record clinical mastitis cases	3	2	1	0	22, 26	0.25
Teat end disinfection prior to intramammary infusion	2	2	0	---	6, 18	0.25
Remove udder hair[5,]*	5	3	1	0	5, 26	0.09
Use DHI records[5]	4	0	1	0	7, 12, 26	0.16
Use CMT*	4	0	0	---	12, 26	0.06
Use bacteriological culture for clinical mastitis cases	3	0	1	0	12, 26	0.25
Age at first calving > 27 mo	1	1	2	1	2, 10	0.38
Minerals fed to pregnant heifers	3	1	1	0	5, 10	0.25
Fed sugar beet pulp to milking cows	1	0	3	2	5, 23	0.25

* Practices demonstrating a consistent association with SCC ($P < 0.15$)

[1] Study identification index number as reported in Table 1; since many studies have compared more than 2 groups of herds, the number of Low SCC and Hi SCC comparisons will not necessarily add up to the number of studies reporting them.

[2] *P*-value of the binomial test of obtaining the observed number of comparisons in a given direction under the null hypothesis of no association between the practice and SCC (one-sided sign test).

[3] Number of comparisons with the mentioned direction of effect.

[4] Number of significant comparisons ($P < 0.05$, multiple comparisons adjusted using the Bonferroni correction) with the mentioned direction of effect.

[5] Noticeable differences between explanatory variable definition and/or comparison groups used among studies.

Table III. Management practices significantly associated with SCC and for which effect estimates were reported only once among 36 published articles.

Low SCC		High SCC	
Management practice	Study	Management practice	Study
Milking procedures		*Milking procedures*	
Number of milkers per shift	Rodrigues et al.,2005	Pre-milking teat disinfection with a foaming product	Barnouin et al., 2004
Number of milkers per month	Rodrigues et al.,2005	Wash only dirty teats before milking	Barnouin et al., 2004
Number of milking units per person	Rodrigues et al.,2005	Wet pre-milking teat preparation (*vs* dry)	Barkema et al., 1998b
Number of cows milked per man per hour	Hutton et al., 1990	Wet pre-milking teat preparation with towels from a bucket	Barkema et al., 1998b
Cow by cow pre-milking preparation and attachment sequence	Barkema et al., 1998b	Lag time ≥ 2 min between beginning of pre-milking preparation and attachment of the unit	Erskine et al., 1987a
Use paper towels (*vs* cloth towels)	Bach et al., 2008	No written milking procedures	Rodrigues et al.,2005
Years of post-milking teat disinfection (PMTD)	Barkema et al., 1998b	Use hired milkers	Bartlett et al., 1992
Teat dip applicator cleaned after each milking	Barnouin et al., 2004	Pre-milking teat preparation time ≤ 30 s	Bartlett et al., 1992
PMTD using chlorhexidine	Erskine and Eberhart, 1991	PMTD using latex barrier with germicide	Erskine and Eberhart, 1991
High producing cows milked first	Hutton et al., 1990	Vacuum not turned off prior to unit removal	Hutton et al., 1991
		High SCC cows and clinical mastitis cases milked with a specific unit	Barnouin et al., 2004
		Milking units rinsed, cleaned, or disinfected before 1st lactation cows are milked	Nyman et al., 2009

Table III. (Continued)

Low SCC		High SCC	
Management practice	Study	Management practice	Study
Milking system			
Minimum vacuum (in kPa) in short milk tube at 2kg/min flow	Barkema et al., 1998b		
Disinfectant used in backflush solution	Hutton et al., 1991		
Housing		*Housing*	
Cubicles rather than bedding packed system	Bareille et al., 1998	Loose straw yard (*vs* other types of housing)	Barnouin et al., 2004
Lying space accessibility	Bareille et al., 1998	Tie-stall or outside lot (*vs* loose housing)	Khaitsa et al., 2000
Manure packed system (*vs* other types of housing)	Wenz et al., 2007	Free access of the herd to an enclosure from the cow shed	Barnouin et al., 2004
Bedding treated with superphosphate (*vs* treated with a drying product)	Bareille et al., 1998	Free access to cow shed from pasture during bad weather	Barnouin et al., 2004
Frequency per d that cubicles are cleaned	Barkema et al., 1998b	Barn design (number of rows of cubicles per pen)	Bewley et al., 2001
Clean bedding area	Bartlett et al., 1992	Quantity of manure in bedding area	Bartlett et al., 1992
Clean water troughs	Goodger et al., 1988	Exercise area of milking cows scrapped ≤ 1 time/d	Barnouin et al., 2004
Newspapers as bedding for milking cows	Wenz et al., 2007		
Stalls' length and width for rubber filled mattress stalls	Fulwider et al., 2007		
Mean size of air inlet per row of cubicles	Barkema et al., 1998b		

Table III. (Continued)

Low SCC		High SCC	
Management practice	Study	Management practice	Study
Peri-partum period		*Peri-partum period*	
Bedding % of dry matter in calving pens (CP)	Hutton et al., 1990	Having clean CP	Barkema et al., 1998b
CP floor slatted	De Vliegher et al., 2004	CP used for sick cows	De Vliegher et al., 2004
No outside area for peri-partum cows (*vs* dry-lot or pasture)	Wenz et al., 2007		
Number of days after calving milk is added to bulk tank	Barkema et al., 1998b		
No outside area for peri-partum cows (*vs* dry-lot or pasture)	Wenz et al., 2007		
Number of days after calving milk is added to bulk tank	Barkema et al., 1998b		
Dry period		*Dry period*	
Cephapirin benzathine used as dry cow treatment (*vs* other dry cow treatment products)	Khaitsa et al., 2000	Dry cows housed in a different location than milking cows (*vs* another area of the same shed)	Barnouin et al., 2004
Teat disinfection before intramammary infusion at dry-off	Barnouin et al., 2004	% of dry cows cubicles with > 10% manure in last meter	Barkema et al., 1998b
Teat disinfection after intramammary infusion at dry-off	Barnouin et al., 2004	% of dry cows with > 30% of udder covered with manure	Barkema et al., 1998b
Years of blanket dry cow treatment	Barkema et al., 1998b		
Use coliform mastitis vaccine	Wenz et al., 2007		
Culling and Conformation		*Culling and Conformation*	
% of herd first lactation cows	Bareille et al., 1998	Monthly % of cows culled for mastitis	Rodrigues et al.,2005

Table III. (Continued)

Low SCC		High SCC	
Management practice	Study	Management practice	Study
Culling and Conformation (continued)			
Cows culled when at least one damage teat	Barnouin et al., 2004	% of cows culled for high SCC	Barkema et al., 1998a
% of cows culled for teat lesions	Barkema et al., 1998a	% of dry cows with udder below hock	Barkema et al., 1998b
% of cows culled for udder shape	Barkema et al., 1998a	≥ 10% cows with udder below hock	Bareille et al., 1998
Cows culled when at least 3 clinical mastitis cases	Barnouin et al., 2004		
Heifer and calf management		*Heifer and calf management*	
Calves separated from dam immediately at calving	Wenz et al., 2007	Heifers checked for mastitis < 2 wks before calving	Barnouin et al., 2004
Heifers checked for mastitis > 8 wks before calving	Barnouin et al., 2004	Heifers moved to confined housing on calving day (*vs* before calving)	Svensson et al., 2006
Frequency at which heifers are checked for mastitis	Lievaart et al., 2007	1st lactation cows calved in group	Nyman et al., 2009
Introduction of heifers to milking cows day of calving (*vs* before calving)	Bareille et al., 2000	1st lactation cows milked in CP during the colostrum period	Nyman et al., 2009
1st lactation cows calved in a CP	Barnouin et al., 2004	Fly problem more important than other years	De Vliegher et al., 2004
End term heifers kept with dry-cows when not on pasture	De Vliegher et al., 2004	Heifers pregnant from AI or bull (*vs* AI only)	De Vliegher et al., 2004
Fly control in heifers on pasture (pour-on or 2 ear tags)	De Vliegher et al., 2004	Use of different types of restraining devices for 1st lactation cows during milking	Svensson et al., 2006

Table III. (Continued)

Low SCC		High SCC	
Management practice	Study	Management practice	Study
Heifer and calf management (continued)		*Heifer and calf management (continued)*	
Deworming all heifers (*vs* some or none)	De Vliegher et al., 2004	Clipping heifers udder long before calving or no clipping (*vs* clipping around calving)	De Vliegher et al., 2004
Nutrition of calves and heifers		*Nutrition of calves and heifers*	
Calves are fed milk replacer	Lievaart et al., 2007	Calves are fed high SCC milk	Barkema et al., 1998b
Deworming all heifers (*vs* some or none)	De Vliegher et al., 2004	Clipping heifers udder long before calving or no clipping (*vs* clipping around calving)	De Vliegher et al., 2004
Type of roughage at weaning	Svensson et al., 2006	Calves are fed milk with antibiotic residues	Barkema et al., 1998b
% of corn silage in transition diet	Bareille et al., 2000	Calves drinking in dirty bucket	Barkema et al., 1998b
Corn silage given at calving and onward (*vs* no corn silage given)	Nyman et al., 2009	Kg of concentrates fed to heifers 11-16 mo of age	Svensson et al., 2006
End term heifers supplemented with other than hay, straw, sugar pulp, or silage during summer	De Vliegher et al., 2004	Protein content and amount of concentrates in transition diet	Bareille et al., 2000
Weeks before calving, heifers are introduced to lactating ration	Svensson et al., 2006	At pasture, heifers drink water from river	Barnouin et al., 2004
Nutrition during transition period			
Lower calcium supply during late pregnancy	Barnouin et al., 2004		
Number of days after calving that max concentrates is fed	Barkema et al., 1998b		

Table III. (Continued)

Low SCC		High SCC	
Management practice	Study	Management practice	Study
Nutrition of milking cows		*Nutrition of milking cows*	
Supplement with minerals during summer	Barkema et al., 1998b	% of forages being corn silage	Bareille et al., 1998
Supplement with NaCl	Barnouin et al., 2004	Feed served outside in an uncovered feeding area (*vs* in freestall or covered outside feeding area)	Smith and Ely, 1997
		Vitamin A-D-E supplementation	Wenz et al., 2007
Nutrition of dry cows			
Supplement with minerals during summer	Barkema et al., 1998b		
Vitamin A or E or Selenium supplementation	Erskine et al., 1987b		
Clinical mastitis management		*Clinical mastitis management*	
Mastitis treatment started when 1 clot of milk is observed at successive milkings	Barnouin et al., 2004	Clinical mastitis suspected when painful udder	Barnouin et al., 2004
Has written treatment protocol for clinical mastitis	Rodrigues et al.,2005	Mastitis detection by checking foremilk for clots	Barnouin et al., 2004
Mastitis treatment done by farmer (*vs* veterinarian involved)	De Vliegher et al., 2004		
Minimal number of antibiotic treatments usually given	Barkema et al., 1998b		
Mastitis cases always frequently milked	Barkema et al., 1998b		
Others		*Others*	
Cows with docked tails in herd	Wenz et al., 2007	% of calving at spring (March to May)	Barnouin et al., 2004
Herd manager follows short courses	Hutton et al., 1990	Cattle (any type) or lactating cows are brought onto operation	Wenz et al., 2007

Table IV. List of management practices having non-significant associations with SCC and for which effect estimates were reported only once.

Practice	Study
Milking procedures	
Mean number of milkers milking on a regular basis	Barkema et al., 1998b
Frequent training program for milkers	Rodrigues et al.,2005
Use of a strip cup	Erskine et al., 1987a
Wash teats (*vs* no wash)	Erskine et al., 1987a
Use disinfectant in wash water hose	Hutton et al., 1991
> half of the teat surface is immersed in post-milking teat disinfectant (PMTD)	Hutton et al., 1991
PMTD using iodophor	Erskine and Eberhart, 1991
PMTD using linear dodecyl benzene sulfonic acid	Erskine and Eberhart, 1991
Fore-strip AND pre-milking teat disinfection AND dry teats AND PMTD	Rodrigues et al.,2005
Total milking duration	Bareille et al., 1998
Mean % of cows restrained during milking	Barkema et al., 1998b
Milking system	
Low line pipelines (*vs* other types of milking system)	De Vliegher et al., 2004
High vacuum (low line > 44kPa, jar > 47kPa, high line > 50kPa)	Barkema et al., 1998b
Milking system vacuum reserve per unit (L/min)	Hutton et al., 1990
Mean vacuum at teat end (in kPa)	Hutton et al., 1990
Minimum vacuum (in kPa) in short milk tube at 3kg/min flow	Barkema et al., 1998b
Use Manometer to test milk vacuum	Hutton et al., 1991
Duration of phases A, B, and C of pulsation cycle	Barkema et al., 1998b
Venting of milking unit claws	Hutton et al., 1990
Use of retrograde shields in short milk tube	Hutton et al., 1990
Use a backflush system	Hutton et al., 1991
Number of months since last milking system update	Rodrigues et al.,2005
Liners life in number of cow-milkings	Hutton et al., 1990
Liners visibly worn	Hutton et al., 1990
Use round teat liners	Hutton et al., 1991
Housing	
Tie stalls and no cubicles facilities for milking cows (compared to other types)	Wenz et al., 2007
Feed-delivery design of the facility	Bewley et al., 2001
Wood product bedding for milking cows	Hutton et al., 1991
Composted manure or straw bedding for milking cows	Wenz et al., 2007
Sand bedding cubicles (*vs* waterbeds)	Fulwider et al., 2007

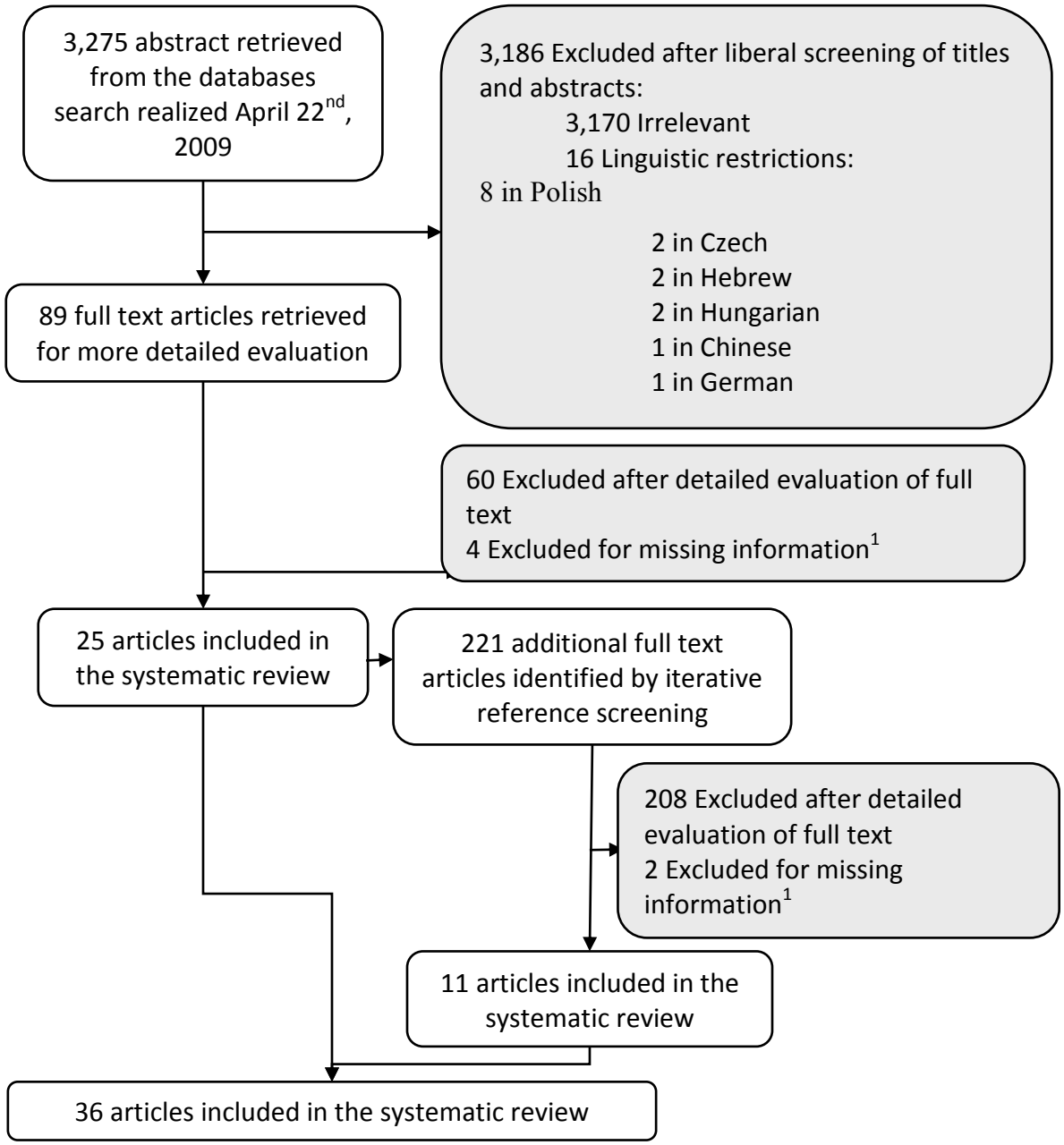
Table IV. (Continued)

Practice	Study
Housing (continued)	
Concrete in milking cows cubicles (*vs* rubber mats)	Nyman et al., 2009
Rubber or concrete in cubicles (*vs* straw or sawdust)	De Vliegher et al., 2004
Depth of bedding in milking cows cubicles	Barkema et al., 1998b
Alley flooring type (concrete, dirt, or rubber over concrete)	Wenz et al., 2007
Alley scrapping frequency	Hutton et al., 1991
Overall ventilation	Bartlett et al., 1992
Use and type of cooling system (fans, sprinklers, both, or none)	Bewley et al., 2001
Manure in exercise area	Bartlett et al., 1992
Cleanliness of exercise area	Bartlett et al., 1992
Wash pen used in summer	Wenz et al., 2007
Peri-partum period	
Calving pens (CP) are used for sick cows	De Vliegher et al., 2004
No concentrates fed during the week before calving	Bareille et al., 1998
Dry period	
Dry cows summer mastitis prevention (ear tag with insecticide)	Barkema et al., 1998b
Dry cows passing through milk parlor before calving	Barkema et al., 1998b
Straw as bedding for dry-cows	Barkema et al., 1998b
Beta-carotene supplementation	Erskine et al., 1987b
Culling	
Overall culling rate	Barkema et al., 1998a
Cull cows based on mastitis alone	Hutton et al., 1990
Heifer and calf management	
Heifers calved on pasture (*vs.* moved to confined housing before calving)	Svensson et al., 2006
Quantity of colostrum fed to calves within first 24 h	De Vliegher et al., 2004
Number of individual calves pens	De Vliegher et al., 2004
End term heifers fed straw during fall/winter	De Vliegher et al., 2004
Vitamin A or E or selenium or Beta-carotene supplementation	Erskine et al., 1987b
Nutrition of milking cows	
Quantity of energy from forage	Bareille et al., 1998
Feeding grass or corn silage	Barkema et al., 1998b
Feeding protein supplements	Barkema et al., 1998b
Fed magnesium oxide	Barkema et al., 1998b
Fed potatoes	Barkema et al., 1998b

Table IV. (Continued)

Practice	Study
Nutrition of milking cows (continued)	
Water from a well in housing season	Barkema et al., 1998b
Feed grain in the milking parlor	Smith and Ely, 1997
Vitamin A or E or Beta-carotene supplementation	Erskine et al., 1987b
Herd mean vitamin E serum concentrations	Weiss et al., 1990
Clinical mastitis management	
Severity of clinical mastitis cases treated	Erskine et al., 1987a
Partial/complete insertion for intramammary infusions	Hutton et al., 1990
Teat disinfection following intramammary infusions	Hutton et al., 1990
Others	
Use culture for suspected subclinical cows	Erskine et al., 1987a
Bulk milk culture several times per year	Rodrigues et al.,2005
Have requested *Mycoplasma* culture	Rodrigues et al.,2005
Almost exclusive use of homegrown heifers as replacement	Hutton et al., 1990
Weaned heifers are brought onto operation	Wenz et al., 2007

Figures



[1] Excluded after multiple unsuccessful attempts to obtain missing information from authors.

Figure 1. Flowchart describing the process of identifying relevant literature on associations between dairy farm management practices and herd somatic cell count.

Development and validation of a bilingual questionnaire for measuring udder health related management practices on dairy farms

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Abstract

Questionnaires are frequently used instruments to collect data in epidemiological studies. In countries where more than one language is spoken, the development of a questionnaire in more than one language is needed. The objective of this study was to develop and test the repeatability and validity of English and French versions of a personal interview-format questionnaire designed to capture udder health related management practices used on dairy farms. A standardized protocol was used to develop and translate the research instrument. Equivalence of the English and French questionnaires was assessed using a cross-over study design with 24 bilingual dairy producers completing both versions on three different occasions in a randomly assigned sequence. Repeatability of the questionnaire was evaluated using the test-retest method with the same questions being asked on two different occasions to 88 dairy producers participating in the National Cohort of Dairy Farms of the Canadian Bovine Mastitis Research Network. Validity of the questions related to milking procedures and general housing was assessed using on-farm observations as a gold standard. Measures of agreement were calculated using Kappa, quadratic-weighted Kappa and concordance correlation coefficients (**CCC**) for categorical, ordinal and continuous variables, respectively. Sensitivity and specificity estimates were computed for the validity analysis. The overall equivalence of the English and French versions of the questionnaire was adequate; agreement measures when administered twice in the same language were not significantly higher than when administered in each language. Similarly, questionnaire overall repeatability was good. When accounting for prevalence bias, Kappa and CCC estimates ranged from 0.40 to 0.92 for 27 of the 29 items evaluated in the questionnaire, with 18 items yielding agreement estimates greater than 0.60. Finally, milking procedures and general housing questions validity was excellent with mean sensitivity and specificity of 86% and 92%, respectively. Although the overall evaluation of the instrument was satisfactory, specific doubtful items were identified. This illustrates the need to address questionnaire reliability as even rigorously designed and pre-tested questions can have poor repeatability or validity. Our results indicate that the

developed English and French questionnaires can be used simultaneously to accurately measure the udder health related management practices used on Canadian dairy farms. This questionnaire is adaptable for use in other developed dairy industry populations. The questionnaire is freely available online at www.mastitisnetwork.org under the "Publications/others" section.

Keywords: mastitis, management factors, cattle, questionnaire, translation, repeatability, validation

Introduction

Questionnaires are frequently used instruments to collect data in epidemiological studies. Like any diagnostic test, questionnaires have intrinsic precision and accuracy. For questionnaires, the term repeatability (the ability of a question to give consistent results on more than one occasion) is often used in place of precision, while validity (the degree to which an answer represent the true state of nature) is used in place of accuracy (Vaillancourt et al., 1991; Slater, 1997). The quality of the procedures used to develop, administer and validate questionnaires can have a tremendous impact on the quality of the data collected. Even well designed pre-tested questions can have limited repeatability or be poorly representative of the process under investigation. Over the years, many concerns have been raised and recommendations made to ensure rigorous development and validation of questionnaires (DelGreco et al., 1987b; Schukken et al., 1989; Vaillancourt et al., 1991; Scholl et al., 1992; Slater, 1997). Still, more often than not efforts are allocated to the development of sophisticated statistical models while no formal evaluation of the quality of the questionnaire-derived data used in these models is realized. Data of unknown quality can only yield results of unknown quality (Gordis, 1979; Schukken et al., 1989).

In many instances, the translation of an already existing instrument or the development of a questionnaire in more than one language is needed (Carlson, 2000;

Markaki et al., 2007; Olde Riekerink et al., 2008). This would be the case for any questionnaire targeting a population where more than one language is spoken, such as the Canadian dairy producers' population where English and French are spoken or the United States dairy workers' population where many Hispanic workers are employed. Although the use of a professional translator might seem at first glance like a valuable option, all cross-cultural research argues against this practice as it will more often than not introduce distortion in the original intent of the instrument (DelGreco et al., 1987a; Chang et al., 1999; Beaton et al., 2000). Alternatively, a formal translation process realized by experts in the construct of the instrument should be used and an evaluation of the cross-language equivalence should be performed to avoid using the differentially misclassified data that can results from poorly translated instruments (Sireci, 1997; Chang et al., 1999).

This study is a part of a larger epidemiological study on the associations between modifiable management practices and the incidence and elimination rates of intra-mammary infection (**IMI**) on Canadian dairy farms. The main objective of this part of the study was to develop and test the reliability of a French and English questionnaire capturing management practices used on a cohort of Canadian dairy farms participating in the National Cohort of Dairy Farms (**NCDF**) of the Canadian Bovine Mastitis Research Network (**CBMRN**). Specific objectives were to assess the cross-language equivalence of the French and English versions of the questionnaire, the repeatability of the questionnaire responses and the validity of a selected subset of questions frequently asked in udder health research.

Materials and methods

Questionnaire development

The method used to develop the questionnaire was an application of a structured process documented by Scholl et al. (1992). First, an extensive literature review was

conducted to identify hypothetical biological pathways through which management practices could influence the incidence and elimination rates of IMI. Efforts were made to identify the possible confounders and effect-modifiers potentially influencing the hypothesized pathways. For each identified pathway, measurable variables representative of the underlying biological process were then selected. Experts in a variety of relevant fields were also consulted to complete this first step. Approximately 300 variables on milking procedures, mastitis control, biosecurity, housing, hygiene, nutrition, attitude of the dairy producer and general management were identified. During the 2-year course of the study, dairy producers participating to the NCDF were visited monthly. These visits allowed for many of the variables to be observed directly on-farm. Also, demographics and production data were obtained from dairy herd improvement programs (DHI). Therefore, only the variables that could not be observed directly on-farm or obtained from DHI data were included in the questionnaire. In an effort to validate some questions frequently asked in dairy research questionnaires, a subset of variables, related to milking procedures and general housing, were selected to be measured both by on-farm observations and by the questionnaire.

There is a vast body of literature on bovine mastitis with many studies using questionnaires (e.g. Barkema et al., 1998; Busato et al., 2000; Peeler et al., 2000; Barnouin et al., 2004; Olde Riekerink et al., 2008). Despite this, to our knowledge no instrument has been published that could be used to measure the common management practices used on dairy farms as they are hypothesized to relate to mastitis prevention and control. An original questionnaire was therefore developed in English with questions formulated to assess each of the previously selected variables. Whenever possible, questions already pre-tested in past studies, obtained from personal communications with their authors, were used. The questionnaire was developed to be administered by personal interview. Since French and English versions of the questionnaire were necessary to collect data in the different populations of Canadian dairy producers, attention was given to develop the English version in a translatable prose. Most questions were semi-closed with multiple choice responses or open-ended questions formulated to record continuous data. Questions

were organized by topic and the questionnaire was divided in two parts to keep the administration time under 30 minutes to avoid deterioration of data quality as the interview lengthens (Rothman et al., 2008). The English questionnaire was then submitted to a reviewing committee and amended.

Questionnaire translation

Brislin's model of translation was used to obtain final French and English versions of the questionnaire (Brislin, 1970). Brislin's original model of translation has been used extensively and is still considered nowadays as the preferred method of translation to obtain an equivalent instrument (McDermott and Palchanes, 1994; Beaton et al., 2000; Carlson, 2000; Yu et al., 2004). This model involves a cycle of three steps. The first step is the forward translation from the original language (English) to the target language (French) by a bilingual person who is an expert in the constructs of the instrument and knowledgeable about how the instrument will be used (Carlson, 2000). Some authors recommend the use of a translator whose mother tongue is the target language as they can reflect the nuance of the language more accurately (Hendricson et al., 1989; Beaton et al., 2000). This first step was accomplished by the main author, a bilingual native French speaker. His udder health expertise as well as his understanding of the content of the instrument and experience with both source and target populations was a key factor to ensure the development of an equivalent French version.

The second step in Brislin's model is backward translation to the original language by a second translator who is blind to the original version. Blinding ensures that the back-translated version reflects no more than the target language version (Brislin, 1970). The blind backward translation from French to English was realized by a trilingual animal health professional raised in a French Canadian community with Czech and English as mother tongues. The two translators conducted their translations independently and no consultation between them was allowed.

The last and perhaps most useful step is the comparison of the original instrument with the back-translated version to detect translation differences. This comparison was performed by the two translators working together. Detailed information concerning translation efforts was then shared between the two translators with special attention to items that were difficult to translate and items about which translators had doubts. The equivalency of the original and back-translated instruments was judged satisfactory and a second cycle of Brislin's translation model with a new team of translators was not deemed necessary. As suggested by Brislin (1970), the back-translated English version was used as the final English version of the questionnaire. Both English and French versions were presented to monolingual expert committees to ensure that all questions could still be easily understood. These monolingual committees were composed of dairy practitioners, dairy researchers, post-graduate veterinary students, and research technicians that worked on a regular basis with NCDF participants. Both committees were satisfied with the questionnaires and no modifications were recommended.

Questionnaire pre-testing

An administration protocol was developed based on Hartge and Cahill recommendations (Rothman et al., 2008) and interviewers were trained to use the questionnaire. Interviewers were the seven research technicians and investigators who had primary responsibility for the NCDF operations in each coordinating center. In this administration protocol, interviewers were instructed to completely read each question as well as the answer choices to the interviewee and to maintain the same tone of voice, especially when reading the different answer choices. If a question was not understood by the interviewee, the interviewers were allowed to give a short clarification such as definition of a term used, but were not allowed to give examples or to favor a specific answer. The interviewers were under no circumstances allowed to help the interviewee select an answer. The questionnaire was pre-tested using the described administration protocol on two French and five English dairy producers living in four different areas of

Canada (Alberta, Ontario, Quebec, and the Atlantic provinces). The duration of administration was recorded and interviewers' and interviewees' comments were collected. Minor adjustments were made following the pre-test: question order was revised, and skip patterns were improved.

Cross-language equivalence test

Although a formal translation process was used, assessing cross-language equivalence is a crucial step, as the equivalence of the original and back-translated English versions does not necessarily imply equivalence between the final English and French versions. Based on a previously described method (Sireci, 1997; Carlson, 2000), a cross-over study design with bilingual dairy producers was used to assess equivalence of the French and English questionnaires. A sample size of 30 producers was estimated sufficient to detect a statistically significant difference between a Kappa of 0.40 and a Kappa of 0.90 with an alpha of 95% and power of 80% (Sim and Wright, 2005). Three large animal veterinary clinics located in known bilingual areas of the province of Quebec were asked to participate by providing the name, address and phone numbers of all their bilingual dairy clients. Letters were sent to the listed dairy producers to inform them of the study and the main author contacted each of them by phone shortly thereafter to inquire about their willingness to participate and to schedule a first interview.

A questionnaire composed of a subset of 51 questions from the original questionnaire was administered in person by the main author to these dairy producers on 3 different occasions. In this instrument, an open question was included to capture any changes that might have occurred between 2 administrations. Answers to this question were used to differentiate a real modification of a management practice between two administrations from a violation of repeatability of the questionnaire. For the first round of data collection, half of the subjects were randomly chosen to answer the French version while the other half answered the English version. Within 2-3 weeks, the questionnaires

were re-administered to the subjects in whichever language they had not previously completed. A third time, 2 weeks later, the subjects answered the questionnaire again in the same language as their first interview. This design was developed to compare agreement in the same language (first and third interviews) with agreement across language (first and second interviews). All interviews were administered according to the protocol previously described. Any clarifications that needed to be provided were given in the same language as the questionnaire being administrated. All questionnaires from dairy producers incapable of completely answering a questionnaire in one language within these administration protocol limits were excluded from the analyses. Due to time restrictions, the cross-language equivalence study was realized in parallel to, rather than preceding, the administration of the questionnaire to our target population.

Repeatability and validity

The NCDF dairy producers were selected beforehand based on their willingness to participate in a 2-year longitudinal study as well as on their geographical proximity to one of the four regional centers of the CBMRN (Calgary, Alberta; Kemptville, Ontario; St-Hyacinthe, Québec; and Charlottetown, PEI). Details of the NCDF selection and recruitment are described by Reyher et al. (2011). The two parts of the questionnaire were administered to the 90 NCDF participants in their language of preference using the described administration protocol in two different interviews 1-2 months apart between November 2007 and April 2008. During this administration, seven questions that were obviously and systematically problematic in respect to the lack of precision of the answer or the dairy producers' interpretation were identified by the interviewers and excluded from the questionnaire evaluation (cross-language equivalence, repeatability and validity) as well as from subsequent studies on the associations between management practices and IMI. These questions were related to common diseases frequency, milking and feeding schedules, and culling protocols. The omitted questions were completely independent of any of the other questions remaining in the questionnaire. The repeatability of the

remaining questions was assessed by a test-retest study on the same population of dairy producers; 6 months following the original administration, an instrument constructed in part from questions repeated from the original questionnaires was administered by the same interviewers. New questions already pre-tested in a previous study (Olde Riekerink et al., 2008) on motivations and knowledge of the dairy producers were intertwined with the originally asked questions. In this instrument, open questions were added to capture any change in milking procedures or housing management that may have occurred after the first administration. The date of any change was also recorded to use the appropriate data for each sampling period in future analyses. Variables for which dairy producers reported a change were omitted from the repeatability and validity analyses. Dairy producers answering the retest questionnaire were uninformed that it was part of a repeatability evaluation. The administration time required to administer this third questionnaire was less than 30 minutes. On this last interview, questions that were related to milking procedures and general housing were observed directly on the farm during milking by the interviewers rather than captured by questionnaire. These observations were used as a "pseudo-gold standard" to assess the validity of these previously asked questions. Questions related to attitudes of the dairy producers, such as meticulousness of the dairy producer and completeness of their record keeping, which were self-answered on a 1 to 10 scale in the original instrument, were this time answered by the interviewers as an external validation.

Statistical analyses

To restrict the possibility of erroneous entry, electronic data entry forms that precluded entry of illogical answers were used to transfer questionnaire data and on-farm observations to a database (Access 2003, Microsoft Corp. Redmond, WA). All variables were then checked for unusual or impossible entries. Double entry of a random sample of 20% of the questionnaires and on-farm observations records was performed to estimate data entry error rate.

For categorical variables, repeatability was assessed using the Kappa statistic. Confidence intervals were estimated using the standard error approach (Sim and Wright, 2005). Since paradoxical values of Kappa may occur because of bias or skewed prevalence, the maximum attainable Kappa based on the marginal total and Byrt's prevalence-adjusted, bias-adjusted Kappa (**PABAK**) were also computed to provide an indication of these effects on the Kappa (Byrt et al., 1993). Repeatability of ordinal variables was measured using quadratic-weighted Kappa with confidence intervals constructed using the standard error approach. Weighted Kappa is a generalization of simple Kappa that uses weights to quantify the relative difference between categories so that it accords greater importance to large difference between ratings. Quadratic weights are proportional to the square of the difference between ratings and will under certain conditions be exactly equivalent to the intraclass correlation coefficient (Maclure and Willett, 1987; Sim and Wright, 2005). The following guidelines were used to interpret Kappa values, while taking into account the effect of bias and skewed prevalence: over 0.75, excellent agreement; 0.4-0.75, fair to good agreement; less than 0.4; poor agreement (Fleiss, 1981). For variables measured on a continuous scale, concordance correlation coefficients (**CCC**) were computed. The CCC measures the distance on the Cartesian plane of pairs of data from the 45^{o} line through the origin, taking into account both precision and accuracy of the measurement. Concordance correlation coefficients were computed by the variance components estimation method using SAS software PROC MIXED procedure (SAS Institute Inc., Cary, NC) with subjects treated as a random effect and replicates as a fixed effect (Carrasco and Jover, 2003). For questions related to attitudes of the dairy producers, the inter-interviewer variability was modeled by adding a fixed covariate term for interviewer in the mixed model. Confidence intervals for the CCC were computed using the Fisher-Z transformation (Lin, 1989). Plots of studentized residuals against their normal score were inspected to detect violation of the assumption of normality of the residuals. Box-Cox transformations of the variables were used to improve the normality of residual distributions when needed (Box and Cox, 1964). Since the extension of CCC to categorical data produces estimates of agreement identical to the Kappa and weighted

Kappa statistics, the previously mentioned guidelines were used to interpret CCC values (King and Chinchilli, 2001). Limits of agreement plots (plot of the difference between pairs of data against their mean, Bland-Altman plots) were inspected to detect noticeable patterns of disagreement between observations to better understand the origin of disagreement and to help categorize some of the variables for future analyses (Dohoo et al., 2003).

The validity of the milking procedures and general housing data obtained from the questionnaire was measured against the on-farm observations made by the interviewers. Sensitivity was defined as the proportion of dairy producers who reported using a specific management practice in the questionnaire among all the dairy producers for whom the interviewer observed this management practice being used on the farm. Specificity was defined as the proportion of dairy producers who did not report using a specific management practice in the questionnaire among all the dairy producers for whom the interviewer did not observed this practice being used on the farm.

Results

Translation and cross-language equivalence

The English and French versions of the instrument can be found in Annexe I and II respectively. In general, the translation process was easily realized with only one term, "kiln-dried", being translated literally. For the cross-language equivalence evaluation, a list of 33 potentially participating dairy producers was provided. Four of them were misidentified as bilingual, one refused to participate, and four failed to answer one of the questionnaires with the allowed minimal same language explanations. These last four interviewees were excluded from the translation study and were not visited further after their exclusion. Therefore, 24 bilingual individuals were available for the cross-language equivalence test; 14 of them spoke English as their primary language while for the

remaining 10 dairy producers, French was their primary language of use. The average time between the first and second interviews was 16 (SD: 5) days and 27 (SD: 3) days between the first and third interviews.

After visual inspection of the distribution of the residuals, five continuous variables were transformed using Box-Cox transformations as described in Table V. Visual assessment of the residuals' distributions indicated an important improvement of their normality and the transformed variables were used for CCC computation.

The vast majority (89%) of the 46 questions used yielded similar repeatability measures when asked twice in the same language compared to a test-retest realized across languages (Table V). Six items produced significantly different agreement results. Overall, the repeatability measures of the questionnaire administered twice in the same language were not significantly higher than when administered once in each language (one-sided paired t test, $p = 0.13$).

Double entry

Double entry of the random sample of questionnaires revealed a data entry error rate of 0.2% (19 data entry errors out of 7,820 entries). The detected errors were corrected in the main database, but the estimated data entry error rate was judged sufficiently low to terminate any additional double entry validation.

Repeatability

Each of the 90 dairy producers participating in the NCDF answered both parts of the questionnaire. Twenty-seven dairy producers, all located in the province of Quebec answered the French version of the questionnaire while the remaining 63 (17 in Alberta, 27 in Ontario, 1 in Quebec, and 18 in the Atlantic provinces) answered the English questionnaire. Two of the NCDF participants were contacted multiple times and were

unavailable for the test-retest study; therefore 88 dairy producers completed the retest questionnaire. The average time between administrations of the two parts of the questionnaire was 45 days (S.D. 50). The retest questionnaire was administered on average 183 days (S.D. 56) following the administration of the second part of the questionnaire. Twenty-four dairy producers (27%) reported a modification of their milking procedures for at least one of the measured variables during the study period. Twenty-nine producers (33%) reported a modification of their housing management during that same period. Box-Cox transformations were used for the same five continuous variables previously transformed during the cross-language equivalence evaluation.

The repeatability results are summarized in Table VI. For two of the measured variables, "keep records of diseases" and "uniform milking routine", the prevalence of a positive rating was very high and the prevalence indexes as described by Sim and Wright (2005) were greater than 80% indicating a possibly biased Kappa. The overall percentage of agreement was 90% for "keep records of diseases" and 89% for "uniform milking routine", contrasting sharply with the lower Kappa values obtained. When accounting for such bias, the test-retest study showed excellent agreement for 9 items, fair to good agreement for 18 items and poor agreement for two items. These two items were "milking system inspection frequency (times/year)" and "bedding completely removed and replaced frequency (times/month)." The limits of agreement plots highlighted a similar pattern of disagreement for these two items, with good repeatability at lower mean values and very poor repeatability at higher mean values. Figure 2 presents the limits of agreement plot for "bedding completely removed and replaced frequency (times/month)".

Validity

Eighty-one of the 90 NCDF participants were visited during milking time on the third interview. The nine dairy producers that could not be visited during milking were NCDF participants that were identified early in 2008 as less compliant participants in

respect to milk sample collection and excluded from further sampling. Validity results are summarized in Table VII. In general, sensitivity and specificity were excellent. However, sensitivity of the questionnaire to capture sawdust or shavings used as bedding was low. When simply trying to detect wood products bedding as a whole without further specification, both questionnaire sensitivity and specificity were higher. There was only fair agreement between dairy producers' self-evaluation and interviewers' evaluation of "completeness of record keeping" with a CCC of 0.40 (95% C.I.: 0.20, 0.57). Agreement between dairy producer and interviewer on "meticulousness of the dairy producer" was poor with a CCC of 0.26 (95% C.I.: 0.06, 0.43).

Discussion

Evaluating the language equivalence, the repeatability and the validity of a questionnaire is no small task and it can easily become unrealisable as the number of questions asked increases. Using the standardized protocol described by Scholl et al. (1992) to identify the measurable variables representative of the hypothesized biological processes helped to limit the number of variables to be measured while ensuring that the important indices were captured. Furthermore, using the theoretical background to identify variables to be measured rather than simply listing all the potentially pertinent management practices that one can think of, can only lead to easier subsequent interpretation of the associations observed. Limiting the questionnaire to variables that could not be observed by other means also reduced the number of questions to validate.

Overall, the results from the questionnaire evaluation were very encouraging. Upon first administration, however, it was noted that a few questions that had not been detected as problematic during the pre-test were obviously and systematically misinterpreted by our interviewees. Following this first administration, it became evident that the precision of the answers for some other questions was clearly insufficient to be used as risk factor measurements in an epidemiological study. In our opinion, the number of interviewees in

our pre-test was insufficient. There is no actual consensus in the literature on the number of individuals required for a pre-test. The usual recommendations are from 5 to 50 individuals that are representative of the target population (DelGreco and Walop, 1987; Vaillancourt et al., 1991). Our choice of only seven individuals was based on the extensive work done beforehand by the reviewing committee both before and after the translation process. These problematic questions were all identified within the first 20 questionnaire administrations indicating that a pre-test on 20 individuals would have been sufficient to reveal them. With further knowledge from such a pre-test, these questions could have been amended rather than excluded from the cohort study.

The number of data entry errors made was lower than what has been reported in similar investigations (Barnouin, 1980; Schukken et al., 1989). It is possible that the restrictive electronic data entry forms used were responsible for this low error rate.

Cross-language equivalence

The formal translation process used to develop the English and French questionnaires apparently produced two generally equivalent instruments. In our study, the apparent disparity between the two populations could essentially be attributed to the language difference, contrasting with the combination of cultural and language differences usually encountered in cross-cultural research. This particularity of our study was certainly responsible for the relative easiness of the translation process and the general equivalence of the instruments. A few items yielded agreement results that were significantly different when asked twice in the same language compared to cross-language administrations. One of these items was a question related to the interviewee's self-evaluated mastitis-related knowledge. Poorer agreement across languages was observed for this question. An important idiom: "…to keep me out of trouble'' was used in the original question. Idioms are a commonly encountered difficulty in translation and finding a wording in the alternative language with the exact same meaning is often challenging (Brislin, 1986;

Chang et al., 1999; Beaton et al., 2000; Yu et al., 2004). For this item, the back-translated version was the exact replicate of the original question, although with further analyses we can suggest that "… to avoid problems'' would be a more accurate back-translation of the French version for this item. The two possible translations seem to have only slightly different meanings, yet this may be sufficient to explain the different interpretations in each language. These findings illustrate that the use of a back-translation process does not guarantee an equivalent translation. The back-translation process should rather be seen as a validity check to ensure a consistent translation, and, in the end, a test of equivalence across languages has to be realized (McDermott and Palchanes, 1994; Sireci, 1997; Chang et al., 1999; Beaton et al., 2000). In-depth analyses of the remaining four items for which a significant cross-language effect was present did not lead to the identification of any other specific translation problems. It is important to note, however, that due to the large number of comparisons made, the probability of observing these five cross-language differences under a global null hypothesis of no differences is greater than the chosen 5% type 1 error threshold. While this can be seen at first as a serious multiple comparisons threat, one must bear in mind that using such an uncorrected alpha can only diminish our ability to demonstrate the equivalency of the French and English version of the questionnaire. Some of the differences observed could have also occurred due to chance alone; this would explain, for example, the better agreement across languages than in the same language obtained for at least one of the items.

Different methods have been described to correctly assess questionnaire equivalence across languages (Sireci, 1997). The two most common are evaluation by monolingual or bilingual expert committees and cross-over design studies with bilingual individuals taking the instrument in both languages, the latter being considered as the most reliable (Carlson, 2000). Our cross-language equivalence study comparing agreement between same and cross-language administrations could be considered as an improved adaptation of this last equivalence testing approach which usually simply measures agreement across language. A common limitation of this technique is the limited number of bilingual individuals available, and our study suffered from this problem. Our initial goal of 30 bilingual dairy

producers was not met and therefore the power of our study to detect item-specific language effects was reduced. Another, limitation of this study design is the possibility for the bilingual individuals to use both languages when responding. A poorly translated question that would have been problematic to a monolingual individual will then be easily understood by a bilingual interviewee and remain undetected (Chang et al., 1999). It is probable, however, that the monolingual reviewing committees used in our study would have detected these questions. Because of our study design, there was a shorter delay between administrations across languages compared to administrations in the same language. This could possibly yield higher agreement results across languages if the shorter delay leads to an improved recall by the interviewees of the previously given answers. Due to the high number of questions asked, a wash-out period of 14 days seemed sufficient to prevent this type of recall bias. Conversely, the third administration of a similar questionnaire could possibly lead to greater memory of previous answers provided in the first two questionnaires. This would then have biased our same language agreement estimates toward higher values, rendering the language equivalence difficult to obtain.

Repeatability and validity

The evaluation of the repeatability and validity of the questionnaire was realized within our target population during the course of the study for which it was designed. An important advantage of using our target population is that we could obtain estimates of repeatability and validity measures that are valid for the population under study. A major drawback, though, is that questions with inadequate repeatability or validity could only be excluded from our future analyses rather than amended.

Overall participation rate in the repeatability study was excellent and repeatability of the different items was generally satisfactory for our purposes. The high participation rate was not surprising since the NCDF participants had already agreed at the beginning of

the follow-up period to answer a series of questionnaires. They were uninformed that this third questionnaire was part of a repeatability study.

Two items yielded insufficient repeatability measures. "Bedding completely removed and replaced frequency (times/month)" was one of them. Disagreement for this item was higher around mean values of 15-30 times/month (Fig. 2). More precisely, the disagreement seemed to be due to seven dairy producers answering once a month on one test and once or twice a day on the other test. Although the question seemed clearly enunciated to target active replacement of the bedding by the dairy producer, it is possible that this concept was confused by the interviewees with passive (i.e. by the cow's movements) emptying of the bedding from the stalls. In addition, it is possible, for example, that bedding is removed and replaced everyday in dirty stalls and in all stalls once per month. Addressing the question in this manner might then produce different answers depending of the interpretation of the interviewee. Further specification of the concept addressed might have proved useful for this question.

Similarly, overall low repeatability with a marked decrease in precision for higher mean values (higher than twice a year) was noted for "milking system inspection frequency". Pairs of observations from three dairy producers had a strong influence on the overall CCC. Exclusion of these observations produced a shift of CCC from 0.16 to 0.44. For these three pairs, confusion between complete inspection of the milking system and quick check-up done by the milking equipment representative on product delivery visits may have been the source of error.

Validity measures of management practices were excellent. It is important to understand, however, that the measured validity of the milking procedures and housing management is a conditional determination of the real validity. A single observation of milking procedures may not be perfectly representative of the milking procedures regularly used on-farm. A more accurate approach would have required numerous observations of the milking procedures over the 2-year cohort follow-up period.

Questionnaire sensitivity was quite low when trying to identify the dairy producers using wood shavings or wood sawdust as bedding. When simply coding answers for wood products bedding as a whole, the sensitivity and specificity were then both adequate illustrating the confusion between sawdust and shavings bedding. This confusion could have resulted from a lack of clear definitions of shavings and sawdust within the questionnaires, or perhaps from farmers switching between both bedding material types without reporting this minor change. Questionnaire sensitivity was similarly low when trying to detect the operations without a maternity pen. The definition of a maternity pen was not provided in the questionnaire and numerous dairy producers considered their far-off dry pens as maternity pens while the interviewers looked rather for a pen strictly intended for calving. This illustrates the need for clear definitions of the terms used in a questionnaire since even what seems like a simple term can be interpreted differently by different interviewees. Adding a glossary with definitions of the important terms used in a questionnaire would be an excellent way to prevent such bias.

An important assumption in our repeatability and validity evaluation is that all the changes made to the NCDF participants' management practices between the original questionnaire administration and the retest were captured by the added open questions in the retest questionnaire. Of course, this assumption is questionable and chances are that these changes were not completely reported. The amount of unreported changes can hardly be measured. In our case, these unreported changes would have been mistakenly considered as disagreement and would have resulted in a lower apparent repeatability or validity for the concerned items.

An important concern related to the measurement of management practices is the duration of time for which the originally collected measurement remains valid. There are many items in the daily management of dairy farms which probably change too often to be used as risk factors (Schukken et al., 1989). Other management practices are more stable over time. Although an unknown proportion of the changes occurring over a 2-year cohort follow-up period are presumably not reported, tracking of the management practice

modifications and adjustment of the data in respect to the reported modifications should still be realized to provide accurate estimates of association in subsequent analyses. This is well illustrated in our study by the high proportion of dairy producers that reported at least one modification of their milking procedures (27%) or housing management (33%) over a 6-month period. To our knowledge, no other publication has reported the proportion of management practices that are modified over time on dairy farms. In our study, the rather high proportion of management practices modifications reported may be due in part to the style of the dairy producers enrolled; dairy producers volunteering for such a long epidemiological study may be more progressive producers and surely have a certain concern in improving udder health on their farm. We could therefore expect them to be keener at modifying the management practices that could possibly improve udder health on their farms.

Finally, the poor correlation between self-reported and interviewers-reported meticulousness of the dairy producer was quite revealing. Attitudes of dairy producers have been shown to be associated with udder health (Barkema et al., 1999; Barnouin et al., 2004; Jansen and Borne, 2008). Accurately measuring such a subjective characteristic is, however, very challenging. It is possible that a self-reported evaluation is biased toward a more socially desirable answer, hence the need for an external measurement of this characteristic. Due to the regular monthly farm visits and accompanying milking procedure observations by the interviewers during the course of the study, the interviewers' evaluation of dairy producers' meticulousness was in our opinion a valid measurement. Although we can not exactly assess the validity of either of the two measurements, we can at least observe that they do not measure a single unique concept. Similarly, there was only a fair correlation between self- and interviewer- reporting of the completeness of farm record keeping. Although these are again personal evaluations, record keeping seemed to be more objectively assessed than general attitudes, and this is reflected by the slightly stronger correlation.

Conclusion

The amended questionnaire accurately and precisely measured the management practices hypothesized to be associated with the incidence and elimination rate of IMI. Furthermore, the translation process resulted in English and French version of this instrument that can be considered equivalent which will permit comparison of the practices among Anglophone and Francophone dairy producers in our study cohort. The results of this study illustrate the need to address questionnaire reliability as even for rigorously designed pre-tested questions, good repeatability or validity can never be assumed. The developed instrument should be of value in future epidemiological studies realized on dairy farms and could serve as the basis for a standardized instrument for udder health epidemiology research in developed dairy industries. Use of such a standardized instrument would increase comparability between studies. The questionnaire is freely available online at www.mastitisnetwork.org under the "Publications/others" section.

Authors' roles

The first author (Dufour) was the lead author of this manuscript and was responsible along with the last author (Scholl) for the realization of the project. The other authors were responsible for the recruitment and follow-up of the NCDF cohort, the data collection, contributed to questionnaire development, and to design of the repeatability and validity tests. All authors were involved in the reviewing of the manuscript.

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Tables

Table V. Measures of repeatability in the same language and across languages obtained from 2 test-retest questionnaires administered to 24 bilingual dairy producers.

Variable	Coefficient of agreement [a]		
	Same language	Cross-language	95% CI [b]
Monitoring and detection			
Monitor individual production at every milking	0.67	0.75	0.38, 0.96
Use California mastitis test regularly	0.90	0.90	0.72, 1.0
Review Somatic cell count (SCC) individual data (see Table 2 for categories) [c]	0.83	0.82	0.43, 1.0
Collect milk samples for bacteriology analyses to detect intramammary infection (see Table 2 for categories) [c]	0.56	0.48	0.09, 1.0
Keep records of diseases	0.68	1.0	0.36, 1.0
Milking procedures			
Uniform milking routine	1.0	0.64	0.00, 1.0 [e]
Written milking procedures	1.0	0.91	0.74, 1.0 [e]
Milking order chronic infections	1.0	0.78	0.36, 1.0 [e]
Technique to keep cow standing after milking	0.73	0.82	0.46, 1.0
Milking system inspection frequency [d,f]	0.66	0.69	0.38, 0.83
Stalls and housing management			
Alley cleaning frequency [d]	1.00	1.00	----
Stalls or pens manure removal frequency [d]	0.92	0.93	0.84, 0.97
Dirty bedding removal frequency [d]	0.85	0.91	0.69, 0.93
Bedding adding frequency [d,g]	0.59	0.50	0.26, 0.79
Bedding completely removed and replaced frequency [d,f]	0.72	0.68	0.35, 0.89
Percentage of calving occurring in maternity pen [d]	0.96	0.94	0.91, 0.98
Maternity pens are grouped pens	0.58	0.58	0.05, 1.0
Number of days in maternity pen before calving [d,f]	0.68	0.66	0.28, 0.88
Number of days in maternity pen after calving [d,f]	0.82	0.89	0.56, 0.94
Clinical mastitis			
Percentage of clinical mastitis cases treated with antibiotics [d]	0.95	0.98	0.89, 0.98

Table V. (Continued)

Variable	Coefficient of agreement [a]		
	Same language	Cross-language	95% CI [b]
Clinical mastitis (continued)			
Important factors in treating decision			
Cow's production, age and genetics [c]	0.55	0.63	0.24, 0.87
Severity of symptoms [c]	0.66	0.73	0.25, 1.0
Need milk for quota [c]	0.89 *	0.70 *	0.80, 0.98
Price of cows [c]	0.53	0.33	0.12, 0.95
Protocol establish with their vet [c]	0.83 *	0.63 *	0.66, 1.0
Intramammary treatments administered using complete insertion	1.0	1.0	----
Vaccinate against coliform mastitis	0.83	0.83	0.51, 1.0
Miscellaneous			
Udder hair management (see Table 2 for categories)	0.78	0.78	0.38, 1.0
Tail management (see Table 2 for categories)	0.75	0.56	0.49, 1.0
Knowledge, belief (Likert scale)			
High SCC cows easy to detect during milking [c]	0.58	0.62	0.31, 0.85
Staph aureus is a stall problem rather than a milking problem [c]	0.30	0.42	-0.07, 0.67
You can not influence causes of IMI [c]	0.31	0.36	0.01, 0.69
I know enough about mastitis [c]	0.69 *	0.43 *	0.44, 0.95
I should do more about mastitis [c]	0.35	0.38	-0.08, 0.78
Motivations, attitudes (Likert scale)			
Individual SCC is important [c]	0.20 *	0.69 *	-0.22, 0.62
Individual SCC considered as elevated [d]	0.33	0.25	-0.09, 0.65
Bulk tank SCC level considered as problematic [d]	0.55	0.73	0.18, 0.78
Importance of the individual SCC in culling decisions [c]	0.72	0.78	0.56, 0.89
Importance of a chronic IMI in culling decisions [c]	0.76	0.70	0.57, 0.95
I worry about cost of high SCC [c]	0.38	0.27	0.05, 0.72
Udder health is important in bull selection [c]	0.85 *	0.63 *	0.73, 0.96

Table V. (Continued)

Variable	Coefficient of agreement [a]		
	Same language	Cross-language	95% CI [b]
Motivations, attitudes (continued)			
How frequently are these resources used to prevent or solved udder health problems			
Veterinarian [c]	0.39	0.30	0.05, 0.74
Dairy herd improvement association (DHI) representative [c]	0.44	0.35	0.10, 0.78
Nutritionist [c]	0.53 *	0.21 *	0.29, 0.77
Milking equipment representative [c]	0.69	0.70	0.47, 0.92
Other dairy producers [c]	0.64	0.58	0.42, 0.86

[a] Coefficient of agreement is Kappa statistic unless indicated otherwise

[b] 95% Confidence interval for Kappa and concordance correlation coefficient for same language administration

[c] Quadratic-weighted Kappa

[d] Concordance correlation coefficient

[e] 95% confidence interval for Kappa and concordance correlation coefficient for cross-language administration

[f] Original variable transformed using $y^*=ln(y+1)$

[g] Original variable transformed using $y^*=(y^{0.5}-1)/0.5$

* Significant difference ($p<0.05$) between same and cross-language administration

Table VI. Measures of repeatability obtained from 2 test-retest questionnaires administered to a cohort of 88 Canadian dairy producers.

Variable	%[a]	Mean (SD)	Kappa	Kmax[b]	PABAK[c]	CCC[d]	95% CI[e]
Monitoring and detection							
Monitor individual production at every milking	53		0.62	0.80	0.61		0.46, 0.78
Use California mastitis test regularly	34		0.51	0.92	0.57		0.32, 0.70
Review somatic cell count individual data			0.55 [f]				0.08, 1.00
The day I receive my report	88						
Whenever I have time	11						
Never	1.1						
If mastitis problem	0.0						
Collect milk samples for bacteriology analyses to detect intramammary infection			0.78 [f]				0.66, 0.90
No	43						
Suspect cows are sampled	32						
All cows sampled once a year	25						
Keep records of diseases	82		0.61	0.78	0.79		0.38, 0.84
Milking procedures							
Uniform milking routine	94		0.25	0.75	0.78		-0.09, 0.59
Written milking procedures	53		0.72	0.86	0.72		0.58, 0.87
Milking system inspection frequency (times/year) [g]		1.2 (0.7)				0.36	0.16, 0.53
Technique to keep cow standing after milking			0.49	0.86	0.60		0.31, 0.66
No technique used	57						
Distribute fresh feed at milking	42						
Lock cows in head-lock after milking	1.2						

Table VI. (Continued)

Variable	%[a]	Mean (SD)	Kappa	Kmax[b]	PABAK[c]	CCC[d]	95% CI[e]
Milking procedures (continued)							
Milking order chronic infections			0.67	0.86	0.76		0.54, 0.81
Milked last	60						
No milking order	25						
Disinfect unit after chronic mastitis cows	8.2						
Milked last and with specific unit	4.7						
Milked with specific unit	2.4						
Stalls and housing management							
Alley cleaning frequency (times/day) (free-stall only)		7.2 (8.2)				0.92	0.84, 0.96
Stalls or pens manure removal frequency (times/day)		3.5 (2.4)				0.67	0.52, 0.76
Dirty bedding removal frequency (times/day)		2.8 (1.6)				0.48	0.31, 0.62
Bedding adding frequency (times/week) [h]		8.6 (6.0)				0.88	0.83, 0.92
Bedding completely removed and replaced frequency (times/month) [g]		2.0 (8.2)				0.34	0.13, 0.51
Percentage of calving occurring in maternity pen [g]		55 (41)				0.83	0.74, 0.88
Maternity pens are grouped pens	34		0.59	0.86	0.63		0.38, 0.81
Number of days in maternity pen before calving [g]		4.6 (6.2)				0.82	0.72, 0.89
Number of days in maternity pen after calving [g]		1.8 (2.2)				0.56	0.36, 0.71
Clinical mastitis							
Percentage of clinical mastitis cases treated with antibiotics		74 (32)				0.64	0.49, 0.75

Table VI. (Continued)

Variable	%[a]	Mean (SD)	Kappa	Kmax[b]	PABAK[c]	CCC[d]	95% CI[e]
Clinical mastitis (continued)							
Important factors in treating decision, scored from 1 (very important) to 5 (not important)							
Cow's production, age and genetics [f]		3.0 (1.7)	0.40				0.21, 0.59
Severity of symptoms [f]		1.3 (0.8)	0.55				0.22, 0.87
Need milk for quota [f]		3.5 (1.5)	0.50				0.34, 0.67
Price of cows [f]		4.3 (1.1)	0.45				0.24, 0.67
Protocol establish with their veterinarian [f]		3.4 (1.5)	0.42				0.23, 0.60
Intramammary treatments administered using complete insertion	22		0.56	0.87	0.68		0.36, 0.76
Vaccinate against coliform mastitis	42		0.86	0.95	0.86		0.75, 0.97
Miscellaneous							
Udder hair management			0.84	0.86	0.89		0.73, 0.96
Clipped only	65						
No hair management	24						
Flamed only	10						
Clipped and flamed	1.1						
Tail management			0.61	0.86	0.66		0.48, 0.74
Clipped only	37						
Tied only	29						
No tail management	20						
Docked only	8.5						
Clipped and tied	4.9						
Clipped and docked	1.2						

[a] First interview prevalence (or proportion of yes for dichotomous answers)

[b] Maximum attainable Kappa based on the marginal total

[c] Byrt's prevalence-adjusted, bias-adjusted Kappa

[d] Concordance correlation coefficient

[e] 95% confidence interval for Kappa or concordance correlation coefficient

[f] Quadratic-weighted Kappa

[g] Original variable transformed using $y^*=ln(y+1)$

[h] Original variable transformed using $y^*=(y^{0.5}-1)/0.5$

Table VII. Validity measures of milking procedures and general housing between the questionnaire and on-farm observations made on 81 Canadian dairy farms.

Variable	%[a]	Se[b]	95% CI[c]	Sp[d]	95% CI[e]
Milking procedures					
Milk in a milking parlor	36	100	88.3, 100	100	93.1, 100
Milkers wear gloves	56	97.4	86.5, 99.5	76.2	47.4, 88.6
Use pre-milking teat dip	66	96.2	87.2, 99.0	96.3	81.7, 99.3
Method of application of the dip:					
Dip	80	100	91.0, 100	100	72.3, 100
Spray	14	100	64.6, 100	100	91.6, 100
Foam	6.1	100	43.9, 100	100	92.3, 100
Forestrip all cows	53	93.0	81.4, 97.6	92.1	79.2, 97.3
Clean teats all cows	99	100	95.4, 100	0.00	0.00, 79.5
Teat cleaning method :					
Dry-wipe	7.7	66.7	30.0, 90.3	95.8	88.5, 98.6
Pre-dip and wipe	60	91.5	80.1, 96.6	90.3	75.1, 96.7
Udder wash solution	24	89.5	68.6, 97.1	96.6	85.1, 99.0
Water	1.3	0.00	0.00, 79.4	98.7	93.0, 99.8
Commercial disinfecting towels	6.4	100	56.6, 100	100	95.0, 100
Teat drying method :					
No drying	10	57.1	25.1, 84.2	96.8	89.1, 99.1
Single-use paper towel	63	97.7	88.2, 99.6	92.3	70.2, 97.9
Reusable cloth towel	27	94.7	75.4, 99.1	98.0	89.7, 99.7
Milking units equipped with automatic take-off	76	96.7	88.8, 99.1	94.7	75.4, 99.1
Stalls and housing management					
Barn type :					
Tie-stall	61	100	92.7, 100	100	89.3, 100
Free-stall	33	100	87.5, 100	100	93.4, 100
Bedding pack	4.9	100	51.0, 100	100	95.3, 100
Stall base :					
Concrete	13	80.0	49.0, 94.3	95.6	87.8, 98.5
Sand	10.3	100	67.6, 100	97.1	90.2, 99.2
Mattress	58	86.7	73.8, 93.7	84.9	69.1, 93.4
Rubber mats	19	73.3	48.1, 89.1	93.7	84.8, 97.5

Table VII. (Continued)

Variable	%[a]	Se [b]	95% CI [c]	Sp [d]	95% CI [e]
Stalls and housing management (continued)					
Bedding type :					
Straw	66	98.1	90.1, 99.7	88.9	71.9, 96.2
Wood products	34	96.3	81.7, 99.3	88.7	77.4, 99.4
Shavings	16	55.0	34.2, 74.2	91.7	81.9, 96.4
Sawdust	25	53.9	29.1, 76.8	83.6	72.9, 90.6
Sand	5.0	75.0	30.1, 95.4	100	95.2, 100
Hay	1.3	100	20.7, 100	97.4	89.9, 99.4
Bedding quantity (≥2cm)	71	87.3	76.0, 93.7	68.2	47.3, 91.2
Alleys are scraped instead of slatted floor (free-stall only)	94	100	34.2, 100	100	88.3, 100
Number of maternity pen :					
0	36	62.1	44.0, 77.3	100	93.1, 100
1	24	79.0	56.7, 91.5	83.9	72.8, 91.0
2	27	81.8	61.5, 92.7	94.9	86.1, 98.3
3	8.6	85.7	48.7, 97.4	91.9	83.4, 96.2
4	3.7	66.7	20.8, 93.9	98.7	93.1, 99.8
5	1.3	100	20.7, 100	98.8	91.2, 99.8

[a] On-farm observation prevalence

[b] Sensitivity

[c] Sensitivity 95% confidence interval

[d] Specificity

[e] Specificity 95% confidence interval

Figures

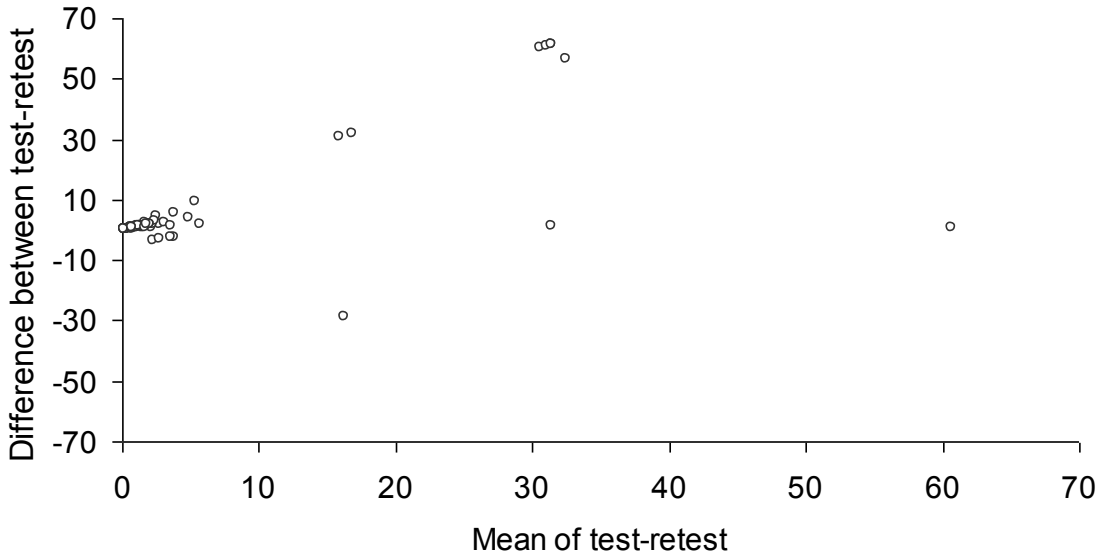

Figure 2. Limits of agreement plot of "bedding completely removed and replaced frequency (times/month)"; obtained from 2 test-retest questionnaires administered to a cohort of 88 Canadian dairy producers.

Manageable risk factors associated with the lactational incidence, elimination, and prevalence of *Staphylococcus aureus* intramammary infections in dairy cows

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Abstract

Staphylococcus aureus intramammary infections (**IMI**) are a major cause of mastitis on farms worldwide. Incidence and elimination rates are the key determinants of prevalence of *S. aureus* and risk factors associated with these rates must be identified, prioritized, and controlled to obtain long-term reduction in prevalence. The objectives of this study were to identify manageable risk factors associated with the lactational incidence, elimination, and prevalence of *S. aureus* IMI. A cohort of 90 Canadian dairy farms was recruited and followed in 2007 and 2008. Quarter milk samples were collected repeatedly from a selection of cows and bacteriological culture was realized to assess incidence, elimination, and prevalence of *S. aureus* IMI. Practices used on farms were measured using direct observations and a validated questionnaire. A linear regression model was used to explore the relationship between herd IMI prevalence and incidence and elimination rates. Multi-level logistic regression models were used to compute measures of associations between practices used on farms and IMI incidence, elimination, and prevalence. The herd incidence rate was the most important predictor of herd IMI prevalence: a reduction of the incidence rate equivalent to its inter-quartile range (0.011 new IMI/quarter-month) was associated with a prevalence reduction of 2.2 percentage-points; in comparison, an equivalent increase of the elimination rate by its inter-quartile range (0.36 eliminated IMI/quarter-month) resulted in a prevalence reduction of 0.4 percentage-points. Post-milking teat disinfection and blanket dry cow therapy were already implemented by most herds. Most of the practices associated with *S. aureus* IMI incidence were related to milking procedures. Among these, wearing gloves during milking showed desirable associations with IMI incidence, elimination, and prevalence. Similarly, adequate teat end condition and use of pre-milking teat disinfection were associated with lower IMI incidence and prevalence. The initial herd prevalence of *S. aureus* IMI was positively associated with subsequent IMI incidence. This indicates that, in some situations, an initial reduction of the pool of infected quarters could be justified. Some housing practices were associated with IMI incidence, elimination, or prevalence. The effects of these latter practices, however, were often

influenced by specific cow characteristics such as parity or days in milk. These results highlight the importance of good milking practices to prevent *S. aureus* IMI acquisition and, therefore, reduce their prevalence.

Key words: dairy cow, mastitis, *Staphylococcus aureus*, incidence, management practices

Introduction

Mastitis is one of the most costly diseases for the dairy industry worldwide. The term mastitis actually encompasses two relatively independent health problems: clinical and subclinical mastitis (Barkema et al., 1998, Olde Riekerink et al., 2008a). Clinical mastitis is an easily observable disease and, for this reason, risk factors associated with prevalence or incidence of clinical mastitis have been the primary focus of much research in the last few decades. Conversely, although SCC can be used to point out potentially infected quarters, accurate identification of subclinical mastitis requires collection and bacteriological culture of milk samples and is, therefore, more complex and expensive to achieve. In addition, detection of new IMI requires repeated milk collection and culture. Because of this latter difficulty, nearly all risk factor studies for pathogen-specific subclinical mastitis have focused on association with IMI prevalence (the proportion of quarters infected at a given point in time) rather than IMI incidence (the number of newly arising IMI per quarter per period of time). Interpretation of associations reported in cross-sectional prevalence studies, however, is complex since the time order of occurrence between the potential exposure and the disease is not known and one can therefore hardly differentiate the cause from the consequence (Rothman et al., 2008).

Despite a decrease in bulk milk somatic cell count in many countries, *Staphylococcus aureus* is still a major cause of subclinical mastitis. The herd prevalence of *S. aureus* as determined by bulk tank culture is still quite high as two recent North American surveys suggest 50-75% of herds have at least one cow with IMI by this contagious pathogen (Anonymous, 2008. Prevalence of contagious mastitis pathogens on U.S. dairy operations, 2007. APHIS Veterinary Services Info Sheet. #N533.1008, Olde Riekerink et al., 2010). Other recent studies have reported cow prevalence of *S. aureus* of 6.4% in a random sample of cows in Switzerland (Moret-Stalder et al., 2009), 3.7% and 15.0% in low and high SCC cows respectively in the Netherlands (Sampimon et al., 2009), and 22.2% in a random sample of cows in Norway (Østerås et al., 2006). The impact of these IMI is considerable; quarters infected with *S. aureus* had a mean geometric somatic

cell count of 333,000 cells/ml of milk (95% CI: 320,000-348,000 cells/ml) compared to 68,000 cells/ml for uninfected quarters (Djabri et al., 2002). In that study, the effect of a *S. aureus* IMI was estimated to be a five-fold increase in SCC. The economic loss associated with subclinical mastitis caused by a *S. aureus* IMI was estimated by Wilson et al. (1997) at $170 USD per lactation.

Some important characteristics of *S. aureus* organisms are their ability to cause persistent IMI and their relatively important resistance to conventional treatments (Barkema et al., 2006). In addition, quarters recovering from *S. aureus* IMI have been shown to have increased susceptibility to reinfection (Zadoks et al., 2002). These characteristics imply that control of *S. aureus* will hardly ever be achieved by treatment of existing IMI. To lower the prevalence of *S. aureus* IMI, manageable risk factors associated with acquisition of these IMI (IMI incidence) have to be identified. Alternatively, manageable risk factors associated with duration or elimination of existing IMI could also be valuable to understand. These latter risk factors could be related, among other things, to improved host resistance to IMI or differential selection of less persistent strains of *S. aureus*.

The study presented is a longitudinal cohort study on naturally acquired *S. aureus* IMI during the lactation on 90 Canadian dairy herds. The objectives were to estimate the effect of manageable risk factors on incidence, elimination, and prevalence of *S. aureus* IMI, taking into consideration the effects of other farm conditions and practices.

Materials and methods

The herds selected were participants in the National Cohort of Dairy Farms (**NCDF**) of the Canadian Bovine Mastitis Research Network (**CBMRN**). A thorough description of the herd selection process and general characteristics of the NCDF herds can be found in Reyher et al. (2011). Briefly, 91 dairy herds were recruited in 2006 in four regions of

Canada based on the willingness of dairy producers to participate in a two year data collection (2007 and 2008). One herd refused to pursue their participation to the NCDF early after the beginning of the study in 2007 because of the amount of work involved. The present study is limited to the 90 herds that contributed to the cohort for at least one year.

Milk Sampling

Series of milk samples were collected on 4 different occasions (referred to as "sampling periods" in the remainder of the manuscript) in 2007 and 2008 (March-May 2007, June-August 2007, January-March 2008, and June-August 2008). At the beginning of each sampling period, a new sample of apparently normal milking cows was selected in each herd (Reyher et al., 2011). This sample consisted of 15 lactating cows: 10 randomly chosen cows and the 5 most recently freshened cows. Cows selected in a previous sampling period were not excluded from the selection process in subsequent sampling periods. During each sampling period, milk from each quarter of the selected cows was collected by a team of trained technicians on 3 occasions at intervals of 3 wks to constitute series of 3 milk samples per quarter. Signs of inflammation of the quarters were noted if present. As part of the CBMRN cohort sampling scheme, NCDF producers were also asked to record clinical mastitis events and submit milk samples for these. During the 2-year course of the cohort study, none of the apparently normal lactating cows selected for the present study suffered from clinical mastitis during the sampling periods. Technicians, however, recorded flakes in the milk without additional signs of inflammation when collecting milk of 72 (0.12%) quarters. These samples were not excluded from the analyses. Teat end condition scores (Neijenhuis et al., 2000) were recorded at the first and last samplings of each series. Bacteriological culture of the milk samples was realized by a uniform protocol that was based on NMC guidelines (Reyher et al., 2011). Briefly, 10 µl of milk was streaked on a Columbia agar +5% sheep blood plate and incubated aerobically at 35ºC for 24 h. The different types of colonies were then enumerated (up to 10 colonies or 10+ colonies) and speciated after 24h using recommended bacteriologic procedures (Hogan et al., 1999) and re-incubated for another 24h. Somatic cell count analysis was realized

using the Fossomatic milk cell counter (Fossomatic 4000 series, Foss Electric, Hillerød, Denmark).

For the organism of interest, *S. aureus*, and for CNS, *Corynebacterium spp*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and streptococci other than uberis, dysgalactiae, and agalactiae (referred to as "other streptococci" in the remainder of the manuscript) presence of an IMI on the day that an individual milk sample was collected was defined as recovery of the organism in concentration of $\geq$ 100 cfu/ml of milk in the associated sample. These definitions were chosen based on the recently reported sensitivities and specificities of various IMI definitions for single milk samples (Dohoo et al., 2011). Based on results from Höfler (2004), the definitions of IMI based on recovery of $\geq$ 100 cfu of a pathogen/ml of milk yielded sensitivity and specificity combinations that would best control the misclassification bias for the different pathogens recovered.

Data Sets

Separate data sets for each of the 3 outcomes of interest (*S. aureus* IMI incidence, elimination, and prevalence) were created. For the incidence and elimination data sets, the series of 3 milk samples collected on a specific quarter during a sampling period were organized in pairs (1st and 2nd samples, 2nd and 3rd samples) and only pairs with complete results were retained. Pairs negative for *S. aureus* on the 1st sample of the pair were considered at risk and determined to have an incident infection if they were positive on the following sample. Pairs that were positive on the 1st sample of the pair were at risk of an elimination which was deemed to have occurred if the subsequent sample was negative.

For the prevalence data set, the series of 3 milk samples collected on a specific quarter during a sampling period were considered as one observation. A prevalent *S. aureus* IMI was defined as series where $\geq$ 1 of the 3 samples collected was found to be positive for *S. aureus*. Series where no samples tested positive for *S. aureus* were defined as free of *S. aureus* IMI.

Explanatory variables

This study was designed to identify risk factors at the herd, cow, and quarter levels. A thorough selection of potential risk factors, confounders, and measure-of-effect modifiers was first performed and is fully described in Dufour et al. (2010). Variables selected were then measured using direct observations and a validated bilingual questionnaire with known sensitivity and specificity (Dufour et al., 2010). The questionnaire was administered on multiple occasions which allowed the identification of any changes in the practices used during the 2-year study and the date at which that change had occurred was recorded. Because of the high proportion of dairy producers reporting at least one modification to their milking procedures (27%) or to their housing management (33%) over a period of 6 months (Dufour et al., 2010), the management practices employed just before and during a specific sampling period were analyzed rather than merely those employed at the beginning of the study. At all times during the analysis, time order of occurrence between exposure and disease was taken into consideration. Pathogen-specific quarter, cow, and herd prevalence of IMI at the first sampling were used as explanatory variables. Dairy herd improvement (**DHI**) data from 2005 to 2009 were obtained for each participating herd to capture data on individual milk production, cow and herd level SCC, and herd demographics. Like questionnaire data, attention was given to DHI data related to the period immediately preceding a sampling period. Contextual effects were taken into consideration during variable selection and construction. Contextual effects can be defined as the effect of a group-level characteristic on an individual outcome and should specifically be investigated whenever the group-level variable provides information that is not captured by the individual-level variable (Diez-Roux, 1998). For instance, the risk of becoming infected with *S. aureus* for a negative quarter belonging to a cow with a high proportion of already infected quarters might differ from that of a quarter belonging to a herd with a high prevalence of infection. In one case (cow prevalence), the effect is theoretically linked to within cow transmission of IMI while in the other (herd prevalence,

the contextual effect) between cow transmission is involved. The prevalence of *S. aureus* in both the cow and herd are, therefore, of interest since they represent different concepts.

The focus of the study was on risk factors that could potentially be manipulated to modulate *S. aureus* IMI incidence, elimination, or prevalence. Consequently, only variables that could theoretically be modified relatively easily were considered as manageable risk factors. For this reason, type of housing system was not considered a manageable risk factor; this variable, as well as other non-manageable variables were included solely as confounders or effect modifiers of the association between manageable risk factors and disease and are referred to as "covariates" in the remainder of the manuscript. Special attention was given to attitudes, motivations, knowledge, and beliefs of dairy producers as potential confounders or effect modifiers of the associations under study. A list of the explanatory variables tested is presented in Table VIII. A full description of the data collection process as well as distributions of the manageable risk factors and covariates in the population under study can be found in Dufour et al. (2010).

Analyses

Data Structure

In all three data sets, since cows could be selected in multiple sampling periods, observations from a cow during a specific sampling period could be cross-classified by herd and by period. In the incidence and elimination data sets, although two pairs of samples per quarter per sampling period were available, the definitions used for quarter at risk of acquiring or eliminating a *S. aureus* IMI precluded any type of correlation between observations collected on a quarter during a sampling period. For instance, a quarter acquiring a *S. aureus* IMI on the first pair would not be considered at risk of acquiring a new IMI for the second pair and, thus, pairs of samples collected on a quarter during a sampling period were truly independent observations. In the prevalence data set, only one observation was available per quarter per sampling period and clustering of observation per quarter was, therefore, nonexistent. In all three data sets, however, observations collected

on a specific cow or on a specific herd during a sampling period were correlated. Structure of the data was, therefore, a hierarchical cross-classified structure with observations clustered by cow and cows clustered both by herd and sampling period. A proportion of 78, 19, 3, and < 1% of the cows in the incidence and prevalence data sets were selected, respectively, for one, two, three, and all four sampling periods; reflecting in part the lower odds of being randomly selected multiple times. In the elimination dataset, 89 and 11% of the cows were selected for one and two sampling periods respectively. The essential part of the correlation structure was, therefore, clustering of observations by cow and herd.

Statistical Analyses

First, the relative impact of herd incidence and elimination rates on the herd prevalence of IMI was assessed. For this purpose, a linear regression model was used. The dependant variable was the quarter prevalence of *S. aureus* IMI in the herd over the 2 yrs of the study and explanatory variables were the herd incidence and elimination rates during that same period.

Then, for each previously described data set, descriptive analyses of all variables were conducted to identify distributions and detect unlikely values. In one herd, pre-milking teat disinfection and gloves were used by half of the milkers. Because of the impossibility to correctly classify this herd for these specific characteristics, observations from this herd were excluded from analyses on pre-milking teat disinfection and use of gloves. Next, in each data set and for each outcome, unconditional associations were estimated to screen the explanatory variables described in Table VIII. To simplify the model building, cross-classification was ignored at this stage of the analyses and a hierarchical logistic regression model which accounted for clustering of observations by cow and herd was used. Hierarchical analyses were performed using the GLIMMIX procedure of SAS 9.2 (SAS Institute Inc., Cary, NC). Parameter estimation was conducted using maximum likelihood (Laplace approximation) whenever possible. Pseudo-likelihood estimation (default estimation method in GLIMMIX) was used whenever

maximum likelihood estimation methods could not yield valid estimates. The general model was as follows:

$$Y_{ijk} = ß_{0ijk} + ß_1 X_{ijk} + v_{0k} + u_{0jk}$$

$$\text{Var}\,(Y_{ijk} \mid \pi_{ijk}) = \pi_{ijk}\,(1-\pi_{ijk})$$

where Y_{ijk} is the odds of acquiring a new *S. aureus* IMI, the odds of eliminating an existing *S. aureus* IMI, or the odds of having a prevalent *S. aureus* IMI, for the incidence, elimination, and prevalence analyses respectively (Y_{ijk} is a function of explanatory variable X through the logit function, and approximately follows a binomial distribution); $ß_{0ijk}$ is the intercept; $ß_1$ is the regression coefficient for explanatory variable X; v_{0k}, and u_{0jk} are the herd and cow random effect respectively and approximately follow normal distributions; π_{ijk} is the probability that $Y_{ijk} = 1$.

For all continuous variables, the linearity assumption was assessed using plots of the log odds of the variable after categorization into quartiles against mid-value of each category (Dohoo et al., 2003). Continuous variables were categorized in a biologically meaningful way whenever the linearity assumption could not be met. Variables with $P \leq 0.20$ (Wald test) were retained as potentially important manageable risk factors or covariates.

Then, for each manageable risk factor retained, a list of variables that could act as potential confounders of the association between this manageable risk factor and the outcome studied was constituted using other variables also retained. To be eligible as a confounder of a given association a variable had to meet three criteria: 1) variable is theoretically associated with the manageable risk factor; 2) variable theoretically affects the outcome under study; and 3) variable is not affected by the manageable risk factor nor the outcome (Rothman et al., 2008). Assessment of the criteria for confounding was realized using principles such as time sequence between variables' occurrence and theoretical biological and sociological relationships between variables. The importance of these selected confounders was then assessed by adding them one at the time to the model

containing the manageable risk factor and by comparing the proportion of change between the adjusted and crude odds ratio as described by Mickey and Greenland (1989). Based on their recommendations, potential confounders associated with a change of ≥ 10% were retained as confounders. Whenever possible, variables used as confounders were left on a continuous scale if continuous even though the assumption of linearity might have deviated from the model assumptions; categories of categorical variables were not collapsed (Brenner and Blettner, 1997). All retained confounders were then added to the model containing the manageable risk factor to provide a confounding adjusted model. Logically plausible effect modifiers were then identified in the list of variables retained by the unconditional analyses. The importance of these potential effect modifiers was tested as follows: the variable and its cross-product with the manageable risk factor were added to the confounding adjusted hierarchical model; a Wald test was realized on the cross-product terms and variables yielding $P \leq 0.05$ were retained as significant measure of effect modifiers.

In order to adjust estimates of association for the cross-classified structure of the data, revised estimates were obtained for each model in MlWin 2.20 (Rasbash, London, UK) using a Bayesian estimation method taking into consideration the complete hierarchical cross-classified structure. Bayesian hierarchical cross-classified models were run with a burn-in period of 500; starting values for each parameter were generated using iterative generalized least square estimation; default MlWin Markov chain Monte Carlo (**MCMC**) sampling methods were used (Metropolis-Hasting sampling with scale factor of 5.8, acceptance rate of 50% and tolerance rate of 10%); they were run for a minimum of 50,000 iterations without thinning. A larger number of iterations was realized when needed to satisfy the Raftery-Lewis diagnostic for the fixed parameters associated with the manageable risk factor and any cross-product terms (Raftery and Lewis, 1992). Visual inspection of the trajectories and posterior distributions of these fixed parameters was realized to insure properly behaving MCMC chains. Assumption of normality of the residuals was checked by visual inspection of plots of standardized residuals against their normal score.

Power of the Study

Approximate power calculations were realized using MLPowSim 1.0 (Browne, Bristol, UK). The sample size for this specific study was bounded by the prearranged CBMRN sampling methodology. Consequently, retrospective power calculations were realized and power of the study is therefore presented in terms of strength of association detectable with 90% power between outcome and a binary explanatory variable located at lowest level of a simplified three level hierarchy (observation, cow, herd), for a range of exposure prevalences, a significance level of 5%, and given the available sample size and observed outcome distribution. For IMI incidence, our study had at least 90% power to detect strength of association greater than odds ratio of 1.6, 1.7, and 2.5 (or, alternatively, smaller than 0.63, 0.59, 0.40) for exposure distribution of 50:50, 70:30, and 90:10 respectively. For IMI elimination, our study had at least 90% power to detect strength of association greater than odds ratio of 2.0, 2.2, and 3.2 (or, alternatively, smaller than 0.50, 0.45, 0.31) for exposure distribution of 50:50, 70:30, and 90:10 respectively. Finally, for IMI prevalence, our study had at least 90% power to detect strength of association greater than odds ratio of 1.6, 1.7, and 2.2 (or, alternatively, smaller than 0.63, 0.59, 0.45) for exposure distribution of 50:50, 70:30, and 90:10 respectively. These power calculations were made with the assumption that exposures and diseases were perfectly measured. In general, more extreme strength of association would be detectable with 90% power for variables defined at a higher level of the hierarchy (cow or herd variables).

Results

Herds selected had a mean of 85 milking cows (range 32 to 326) and mean 305-d milk production of 9,781 kg of milk (range 7,734 to 12,377). Over the 2 year course of the study, 59,167 quarter milk samples were collected in the selected sampling intervals.

Sixty-seven samples were lost or damaged before bacteriological culture could be realized. *S. aureus* was cultured from 1,504 (2.5%) of the 59,100 quarter-milk samples for which culture results were available. Distributions of herd *S. aureus* IMI incidence rate, elimination rate, and prevalence are presented in Figure 3. One striking feature was the skewness of the incidence rate distribution with 92.2% of the NCDF herds having an incidence rate ≤ 0.02 new *S. aureus* IMI/quarter-month and only a few herds having an incidence rate > 0.02, but up to 0.08 new IMI quarter-month. The IMI prevalence distribution showed a very similar but less striking skewed pattern. The IMI elimination rate distribution, on the other hand, was skewed, but was closer to the normal distribution with a very wide range of elimination rates and a less striking resemblance with the IMI prevalence distribution. Results from unconditional analyses are presented in Table IX as supplementary material.

Impact of Incidence and Elimination Rates on IMI Prevalence

Both incidence and elimination rates were significant predictors of the herd *S. aureus* IMI prevalence ($P \leq 0.05$). Scatter plots of the relationships between herd IMI prevalence and herd IMI incidence and elimination rates are presented in Figure 4. The herd incidence rate was the most important predictor of IMI prevalence: a reduction of the incidence rate equivalent to its inter-quartile range (0.011 new IMI/quarter-month) was associated with a prevalence reduction of 2.2 percentage-points; in comparison, an equivalent increase of the elimination rate by its inter-quartile range (0.36 eliminated IMI/quarter-month) resulted in a prevalence reduction of 0.4 percentage-point.

IMI Incidence

The incidence data set comprised 37,919 pairs of milk samples at risk of becoming infected. These pairs were obtained from 15,337 quarters belonging to 3,911 different cows from the 90 studied herds. An incident *S. aureus* IMI was identified in 305 of the pairs at risk, yielding a mean incidence rate of 0.012 new *S. aureus* IMI per quarter-month (95% CI: 0.011, 0.013). The herd IMI incidence distribution was right skewed with a large

proportion of herds experiencing relatively low incidence (Figure 3). The median herd incidence was 0.007 new IMI per quarter-month. Bayesian conditional estimates of association for manageable risk factors that were significantly associated with odds of acquisition of a *S. aureus* IMI are presented in Table X. The manageable risk factors retained were mainly milking procedures. Wearing gloves during milking and using pre-milking teat disinfection were associated with lower odds of acquiring a *S. aureus* IMI. Furthermore, the protective effect of wearing gloves was increased on uninfected quarters of cows that already had other *S. aureus* infected quarters. Extreme callosity roughness and thickness of the teat end was associated with greater odds of IMI acquisition. The herd initial prevalence of *S. aureus* IMI was positively associated with the odds of a IMI acquisition.

IMI Elimination

The elimination data set comprised 958 pairs of milk samples. These were obtained from 586 quarters belonging to 437 different cows from 79 herds. *S. aureus* was not cultured on the second sample of the pair for 264 of the 958 pairs, yielding a mean elimination rate of 0.39 eliminated *S. aureus* IMI per infected quarter-month (95%CI: 0.35, 0.43). The distribution of herd *S. aureus* IMI elimination rate was slightly right skewed around a median herd elimination rate of 0.39 eliminated IMI per quarter-month. (Figure 3). Conditional estimates of association for manageable risk factors that were significantly associated with the odds of elimination of an existing *S. aureus* IMI obtained from the final models are presented in Table XI. Few manageable risk factors were significantly associated with *S. aureus* IMI elimination. Wearing gloves during milking was associated with higher odds of IMI elimination. The effect of stocking density on odds of IMI elimination was different for cows in the first 60 d of lactation compared to cows in a more advanced stage of lactation. Having a culling protocol where > 3 clinical mastitis events are needed before culling a cow was associated with lower odds of IMI elimination compared to not having any defined culling protocol for clinical mastitis.

IMI Prevalence

To constitute the prevalence data set, 20,078 series of quarter milk samples were available. These series were obtained from 15,943 quarters belonging to 3,999 different cows in the 90 studied herds. *S. aureus* was retrieved in at least one sample in 777 of these series, yielding a mean quarter prevalence of *S. aureus* of 3.9% (95%CI: 3.6, 4.2). The herd *S. aureus* IMI quarter prevalence distribution was right skewed with a median herd quarter prevalence of 3.3% and with a relatively small proportion of herds showing high prevalence of infection (Figure 3). Conditional estimates of association for manageable risk factors that were significantly associated with odds of a prevalent *S. aureus* IMI are presented in Table XII. Many manageable risk factors were associated with *S. aureus* IMI prevalence. Similar to the incidence models, wearing gloves, using pre-milking teat disinfection, and adequate teat end condition were all associated with lower IMI prevalence. Higher odds of having a prevalent *S. aureus* IMI were observed in herds where the vacuum level was checked daily. Measuring milk conductivity during milking was associated with higher IMI prevalence in herds housed with free-stalls. Many housing variables were associated with IMI prevalence, but, for many of them, the association observed was modulated by cow's parity. For instance, the type of stall base was not significantly associated with IMI prevalence in first parity cows. Stalls filled with sand or made of concrete were associated with lower IMI prevalence in older cows (>1st parity) compared to stalls covered with a mattress or rubber mat. Similarly, using a depth of bedding $\geq$ 2cm and using longer stalls (>1.9m) were both associated with lower IMI prevalence in older cows (>3rd parity). Having a higher number of maternity pens on the farm was associated with lower odds of having a prevalent *S. aureus* IMI. Having a culling protocol for clinical mastitis requiring >3 clinical mastitis events in a lactation before a culling decision is made was associated with higher IMI prevalence compared to not having any culling protocol for clinical mastitis. Finally, in herds where a veterinarian was not consulted regularly for udder health issues, clinical mastitis treatment duration of more than 2 days was associated with lower IMI prevalence.

Discussion

This is the first study reporting *S. aureus* lactational IMI incidence rate, elimination rate, and the risk factors associated with acquisition and elimination of these IMI in a large sample of herds over such a long period of time. Intramammary infection incidence and elimination rates are the main determinants of the herd IMI prevalence and give better insight into the dynamic of IMI within a herd than IMI prevalence alone. In our study, quarter *S. aureus* IMI prevalence was quite variable between herds (range: 0 to 18.3%). Other recent studies on *S. aureus* IMI prevalence have also observed important herd prevalence variation with herd quarter prevalence ranging from 0 to 40.3% in Belgium (Piepers et al., 2007), and from 0 to 18.6% in Switzerland (Moret-Stalder et al., 2009). With such a range of quarter prevalence between herds, a similar variation in herd incidence and elimination rates of *S. aureus* IMI was expected. The shape of the distribution of herd IMI incidence, still, was quite revealing. In this study most dairy herds were very efficient at preventing acquisition of new *S. aureus* IMI; only a minority of herds experienced an IMI incidence higher than 0.02 new IMI/quarter-month (Figure 3). Furthermore, as expected for a chronic type of IMI such as that caused by *S. aureus*, the rate of acquisition of new IMI had a much greater impact on IMI prevalence than the elimination rate; hence the need to identify the manageable risk factors associated with IMI incidence to decrease prevalence of *S. aureus* IMI.

Manageable Risk Factors

To fully grasp the web of relationships between the manageable risk factors identified and the three outcomes studied, a conceptual chart of these associations based on results obtained from the conditional analyses is presented in Figure 5. Similar to previously published studies on IMI prevalence, numerous manageable risk factors were significantly associated with odds of having a prevalent *S. aureus* IMI. It is important to

realize, however, that these risk factors can only modulate the IMI prevalence through their effects on IMI incidence or elimination. For this reason, less consideration should be given to variables associated solely with IMI prevalence. The results presented in Tables X, XI and XII should instead be interpreted as a whole using the conceptual chart presented in Fig. 5 and more weight should be given to variables associated with many of the outcomes. Among these, having milkers wear gloves during milking was the only identified risk factor in this study that showed desirable associations with all three outcomes. Interestingly, the protective association between wearing gloves and incidence of IMI seemed to improve as the number of already infected quarters on a cow increased. This improvement was, however, limited to the relatively low proportion of uninfected quarters belonging to cows with two or three already infected quarters and could actually have been modulated by a third extraneous variable or could have occurred by chance alone. Previous studies have shown that transmission of *S. aureus* IMI can arise from infected herd mates but also from infected quarters on the same cow and that these two processes operate on different scales (Østerås et al., 2006, Zadoks et al., 2001). In this study, wearing gloves during milking seemed to be an efficient technique to prevent IMI transmission from infected herd mates, as can be concluded from the significantly lower odds of IMI acquisition observed for quarters of cows without any *S. aureus* infected quarters. In addition, wearing gloves seemed to prevent transmission of IMI from other infected quarters of the same cow, as can be seen from the generally decreasing odds of IMI acquisition associated with wearing gloves as the number of infected quarter on a cow increases. Although wearing gloves is a commonly recommended practice and has been found in a recent literature review to be consistently associated with lower SCC (Dufour et al., 2011), there is little evidence of the exact mechanisms through which gloves may limit transmission of contagious infections. Wearing gloves during milking is traditionally recommended in part to prevent colonization of milkers' hands with transient flora such as *S. aureus,* and, therefore, to prevent subsequent transmission to uninfected quarters. Evidence that colonization of the skin of gloved hands with *S. aureus* occurs (Doebbeling et al., 1988), however, casts doubt on the rationale behind this principle. In addition, that the preventive effect of wearing gloves

increased as the number of already infected quarters of a cow increased, as observed here, can hardly be explained by this underlying principle. On the other hand, Olde Riekerink et al. (2008b) found that lower bacterial counts were retrieved on gloved hands compared to bare hands after milking. It seems plausible, therefore, that the physical properties of gloves are an important limiting factor for bacteria adhesion, and that wearing them might greatly reduce the potential role of milkers' hands as vector for transmission of *S. aureus* IMI. Finally, having milkers wearing gloves can also potentially increase milkers' awareness towards hand hygiene in general and, hence, reduce the amount of bacteria presented to the teat end.

In this study, wearing gloves during milking was also associated with higher odds of elimination of an existing *S. aureus* IMI. It is speculated that wearing gloves during milking might have efficiently restricted the transmission of the contagious, well host adapted, and persistent strains of *S. aureus* in these herds. The strains found in these herds may, therefore, have originated in a greater proportion from non-mammary sites and were possibly less well adapted to the mammary gland, hence their higher elimination rate. Previous studies have reported the relatively important genetic variability between *S. aureus* isolates retrieved in a given herd (Middleton et al., 2002, Zadoks et al., 2000). A certain variability between herds in the number of different strains present has also been reported and has been associated with specific management practices (Middleton et al., 2002). In addition, Roberson et al. (1994) demonstrated that *S. aureus* appears to be omnipresent in the environment of cows, and that the environment could be a potential source of *S. aureus* IMI. The association between gloves and odds of eliminating an existing IMI could, therefore, possibly be mediated by the initial differential selection of *S. aureus* strains causing IMI rather than by any authentic increased cure rate. Nevertheless, the significant associations seen with all three IMI outcomes in this study and with IMI prevalence in many previous studies indicates that wearing gloves during milking is a crucial component of any *S. aureus* control program.

Similarly, using pre-milking teat disinfection was associated with lower *S. aureus* IMI incidence and prevalence. Pre-milking teat disinfection is usually recommended as a means to prevent environmental IMI (Nickerson and Boddie, 1997). Oliver et al. (1993), however, previously reported lower incidence of *S. aureus* IMI on quarters where teats were pre-dipped and post-dipped compared to quarters where teats were only post-dipped. In addition, Piccinini et al. (2009) observed that some *S. aureus* strains were isolated both from the teat skin of cows and from milk of infected quarters, highlighting the possible role of teat skin contamination and, by extension, of teat skin disinfection in *S. aureus* IMI epidemiology.

In this study, increasing callosity and roughness of the teat end were generally associated with higher odds of IMI acquisition and, consequently, with higher IMI prevalence. The healthy teat end serves as the primary barrier hampering bacterial penetration into the mammary gland. Teat end lesions and callosity have been associated previously with higher prevalence of subclinical mastitis (Sieber and Farnsworth, 1981) and higher incidence of clinical mastitis (Neijenhuis et al., 2001). The development of a small amount of teat end callosity can be seen as a normal physiological and desirable adaptation that follows initiation of milking; the building of an extremely thick or rough callosity, on the other hand, can impair teat sphincter function and favor penetration of bacteria into the mammary gland. Prolonged milking time and practices resulting in extended milking duration have been associated with increased degree of lesions and increased teat end callosity roughness (Farnsworth, 1995, Neijenhuis et al., 2000). Having proper teat stimulation, an appropriate lag time between teat stimulation and milking unit attachment, and using well-adjusted automatic take-offs are all key conditions that can reduce milking duration and prevent the formation of extreme callosity, thereby potentially reducing *S. aureus* IMI incidence. In the present study, however, measurements of the function of the milking system and of milking procedure timeliness were not captured, and, therefore, a direct link between IMI incidence and these important parts of the milking procedures could not be made.

Measuring milk conductivity to detect cows suffering from clinical mastitis was associated, in this study, with higher incidence and prevalence of *S. aureus* IMI. This practice was used in only six of the participating herds; one herd housed in tie-stalls, one in a bedded-pack, and 4 herds in free-stalls and was associated with higher odds of IMI acquisition in general and with higher odds of having a prevalent IMI in herds where a free-stall system was used. It is probable that the associations observed between this practice and *S. aureus* IMI outcomes are consequences of another latent unmeasured concept rather than causal associations (residual confounding). For instance, dairy producers with specific attitudes, motivations, or knowledge might be more inclined to adopt such technology and these social traits have be shown to be associated with IMI epidemiology (Jansen and Borne, 2008). Although none of the social traits measured in this study could be identified as important confounders of this association, we must underline that the social traits measurements were shown to carry a relatively high level of measurement error (Dufour et al., 2010). While practices used on farm are likely to stay relatively constant over time, attitudes, motivations, and knowledge tend to vary and evolve from day to day. Any adjustment for these imperfectly measured confounders would then be an incomplete or inadequate adjustment.

Similarly, higher odds of having a prevalent *S. aureus* IMI were observed on farms where dairy producers verified vacuum level of the milking system at least daily. Again, the observed association could be resulting from residual confounding by social traits or any other unmeasured or imperfectly measured factor. In this case, though, the only significant association was with prevalence of infection and, like numerous prevalence studies prior to this one, reverse order of causation cannot be ruled out. In fact, it is probable that dairy producers that have a high prevalence of *S. aureus* IMI and are aware of that fact might be more inclined to increase their level of surveillance of the milking system rather than the other way around. The observed association illustrates an important issue that needs to be dealt with when analyzing prevalence data arising from cross-sectional study designs.

The herd prevalence of *S. aureus* IMI at the beginning of a sampling period showed a strong positive association with the subsequent odds of acquisition of an IMI. For each 5% increase in the herd proportion of *S. aureus* infected quarters, the odds of subsequent IMI acquisition were increased twofold (Table X). Furthermore, uninfected quarters from cows having one, two, or three already infected quarters had respectively 4.5, 6.8, and 15.8 higher odds of becoming infected compared to quarters from cows without any infected quarters (Table IX, supplementary material). Similar to the work of Middleton et al. (2001), these results suggest that, in herds experiencing a relatively high prevalence of *S. aureus* IMI, reducing the pool of infection through culling, permanently drying off, or successfully treating infected cows or quarters may, in some situations, be an important step to control the subsequent rate of acquisition of new IMI. In these herds, reduction of the pool of infected quarters, coupled with other measures aimed at preventing transmission of IMI from infected to uninfected quarters, would potentially be required to match the infectious pressure power from the collection of infected quarters. The full benefit from culling infected cows or drying off infected quarters, however, would be lost if the time between the initial infection of a quarter and the subsequent culling or drying decision is too long (White et al., 2006). This difficulty would limit the practicality of this control measure in some herds. In addition, in herds with moderate to low prevalence of IMI, although an initial prevalence reduction may reduce the subsequent incidence, it may not be the most economic option.

Although *S. aureus* is mainly recognized as a contagious pathogen that can be transmitted during milking, many housing related variables were associated with IMI prevalence. Again, since nearly all of these variables were associated strictly with the prevalence outcome, the credibility of these results should be questioned. Nevertheless, these housing variables could possibly have an effect on IMI prevalence by altering the cow's defenses against infection, increasing or decreasing contact between bacteria and the teat end, or promoting or reducing the growth of specific strains of *S. aureus* in the environment of the cow. A noteworthy observation is that the effect of most housing variables varied depending on the cow's characteristics, primarily parity, but also days in

milk (see Figure 5). This seems to underline the important effect of the interface between host and environment in IMI epidemiology and, more precisely, of the adaptation capability, or lack of adaptation capability of specific groups of cows to their environment. The associations observed could, therefore, be mediated rather by an alteration of the cow's immune defenses. These fragile observations should be further investigated and, if confirmed, would support the design of housing conditions specific to particular groups of cows, so that the environment is adapted to the cow rather than the opposite. These results would suggest, for instance, that older cows in particular could benefit from the increased comfort provided by longer and more comfortable stalls.

Increasing the number of maternity pens was associated with lower odds of having a prevalent *S. aureus* IMI. Cows usually spent a limited amount of time in a maternity pen at the beginning of the milking period, and the vast majority of cows followed in this study had already joined the regular milking herd when first sampled. Maternity pen variables, therefore, were not tested as possible risk factors for incidence nor elimination of IMI during the milking period since cows were not exposed anymore to these factors. Nevertheless, the early milking period is recognized as a period of tremendous importance for acquisition of new IMI. To better understand the association observed between number of maternity pens used and IMI prevalence during the milking period, studies specifically evaluating the impact of housing around calving time on the incidence of IMI during the early lactation should be conducted. Such studies could shed light on some of the results obtained from those already conducted on risk factors associated with prevalence of IMI during the early lactation (Nyman et al., 2009, Svensson et al., 2006).

In free-stall housing systems, having a cow:stall ratio $\geq$ 105% was associated with lower odds of eliminating an existing IMI in early lactation followed by statistically significantly increased odds of elimination for cows over 60 DIM. The number of observations available to obtain this estimate, however, was limited resulting in a large confidence interval around the estimate. One of the generally recognized consequences of overstocking is to reduce the cow's immune function through increased stress caused by

competition for stalls and feed bunk access (Fregonesi et al., 2007, Huzzey et al., 2006). It is possible that during the early lactation period, the increased stress caused by overstocking would further compromise the already depressed immune functions of the cow, leading to acquisition of IMI that are not necessarily well host-adapted and that are less persistent. As the immune system of the cow returns to normal later on during lactation, these less persistent IMI would then easily be eliminated, explaining the observed increased elimination rate.

Finally, management of clinical mastitis cases was associated with some of the *S. aureus* IMI outcomes. In herds where the veterinarian was not consulted regularly concerning mastitis problems, longer treatment duration (> 2d) for clinical mastitis cases was associated with lower odds of having a prevalent IMI. Researchers have reported higher cure rate of both subclinical and clinical *S. aureus* mastitis with longer treatment period (Barkema et al., 2006). This association, however, might also be indicative of the effect of the dairy producer' motivations and attitudes toward mastitis rather than a true effect of treatment duration. Actually, producers that treated clinical mastitis cases for longer duration may have had a more conscientious attitude toward mastitis in general and be more motivated to improve udder health in their herd. In addition to treatment duration, choice of culling protocols regarding cows with multiple clinical mastitis episodes in a given lactation showed conflicting associations with *S. aureus* prevalence and elimination rate. As expected, odds of eliminating a *S. aureus* IMI were lower for dairy producers waiting for more than three clinical mastitis episodes before culling a cow, suggesting that this practice can lead to persistent IMI. Alternatively, odds of having a prevalent *S. aureus* IMI were lower in these herds. Again care must be taken when interpreting prevalence data, as the explanation for the association observed could well be that dairy producers who had a low herd *S. aureus* IMI prevalence did not need to use a strict clinical mastitis culling protocol rather than the other way around.

In this study, some of the practices that have been recommended for years in mastitis control programs such as universal dry cow therapy and post-milking teat

disinfection were not significantly associated with any of the three outcomes studied. It is important to mention, however, that these practices were widely adopted by the participating herds; 88% of the herds used universal dry cow therapy and 99% used post-milking teat disinfection. The power to identify a significant association between these practices and our IMI outcomes was therefore quite low. These practices have shown very desirable and consistent associations with udder health in general (Dufour et al., 2011) and should, therefore, not be ruled out of mastitis control programs based on results from this specific study. The practices identified in this study should instead be considered as practices that could potentially further improve the udder health situation in herds where post-milking teat disinfection and blanket dry cow therapy have already been implemented.

Potential Biases

Like all previously published udder health studies, many potential biases might be operating in this study and may have influenced the observed results. First of all, herds selected to participate in this study were not a random sample of Canadian dairy herds. Efforts were made, though, to recruit a selection of herds that would be as representative as possible of the Canadian dairy industry and a comparison of the selected herds from this cohort with Canadian herds realized by Reyher et al. (2011) suggest that selection was valid in terms of number of milking cows, milk production, type of housing system used, and geometric and arithmetic SCC averages. Nevertheless, as previously discussed in Dufour et al. (2010), the dairy producers volunteering for such a study might be more progressive and the management practices employed on their farms might differ from those of other Canadian dairy farms. In addition, regular visits by a team of technicians and researchers might have promoted the use of some management practices by the NCDF producers. Any resulting bias would most likely be reflected in the estimates of incidence and elimination rates, of prevalence, and of proportion of herds using specific management practices. This bias would be much less likely to adversely affect the observed relationships between risk factors and outcomes of interest.

Secondly, although attempts to adjust for the most important confounders were made, it is likely that some residual confounding may still bias the observed results to some extent. This residual confounding could arise from unknown unmeasured confounders and from imperfectly measured confounders. With an important confounder being ignored, reverse causality may have occurred despite the care taken to ensure the logical temporal relationships between variables. The direction of bias caused by this lack of adjustment is unpredictable. The magnitude of these biases, however, is probably relatively small. Numerous studies related to udder health and to *S. aureus* in Canada and in other parts of the world have been conducted in the last few decades, and it is very unlikely that a strong confounder of the association between practices used on farm and *S. aureus* IMI would not have been identified before. Similarly, adjustment of an association with an imperfectly measured confounder such as dairy producers' attitudes and motivations would provide an incomplete but still significant adjustment.

Finally, and most importantly, none of the outcomes assessed or factors evaluated were free of measurement error. For instance, *S. aureus* infected quarters were identified using routine bacteriological culture which is known to have imperfect sensitivity (Dohoo et al., 2011). It might be assumed that these measurement errors are probably occurring randomly (non-differential). Under this assumption, the resulting bias direction is predictable and toward the null value, and the associations reported are therefore not as extreme as the true associations. The major impact of this bias, therefore, would be a reduction of power of the study. Fortunately, with the high reported sensitivity of *S. aureus* bacteriological culture (90%) and specificity of nearly 100% (Dohoo et al., 2011), the magnitude of this bias would be relatively small. In this study, in addition to misclassified outcomes, a certain level of exposure and covariate misclassification has been reported (Dufour et al., 2010). As with outcome misclassification, under the assumption of non-differential exposure misclassification, a predictable bias toward the null would be expected. Covariate misclassification would reduce the ability to remove confounding effects and, as noted above, this bias might be in either direction, but would likely have been quite small. Overall, it is most likely that measurement error biases would have biased

observed results toward the null so the true effect of factors evaluated are likely to be greater than those reported in this manuscript.

Conclusions

Preventing acquisition of new *S. aureus* IMI seems to be the key determinant in control of *S. aureus* IMI prevalence. In herds where post-milking teat disinfection and blanket dry cow therapy have already been implemented, there are many additional practices that can be used to further modulate IMI incidence; most of these practices are related to milking procedures. In herds experiencing a very high prevalence of *S. aureus* IMI, a first round reduction of the number of infected quarters may possibly be needed to complement measures aimed at preventing new IMI acquisition.

Authors' roles

The first author (Dufour) was the lead author of this manuscript and was responsible along with the second (Dohoo) and last author (Scholl) for the realization of the project. The other authors were responsible for the recruitment and follow-up of the NCDF cohort, the data collection, and contributed to the design of the study and analyses. All authors were involved in the reviewing of the manuscript.

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Tables

Table VIII. Summary of explanatory variables analyzed for association with *Staphylococcus aureus* IMI on 90 Canadian dairy herds, 2007-2008.

Manageable risk factors	
1.Milking procedures	Uses uniform milking routine, has written milking procedures, milkers receive bonus for milk quality, milker training frequency, milkers wear gloves, gloves are cleaned during milking, cows are fore-stripped prior to milking and placet where foremilk is discarded, teats cleaning and drying methods, number of cows per cloth, uses pre- and/or post-milking teat disinfection and type of application and frequency of cleaning of applicators, milks cows with high SCC, *Staphylococcus aureus* IMI, or clinical mastitis last or with a specific milking unit, milker:cow ratio, milker:milking unit ratio, cow:milking unit ratio, owner milks cows, uses a technique to keep cows standing after milking, teat end condition
2.Milking equipment	Type of milking system, use of automatic take-off, milking system inspection frequency, vacuum check frequency, teat cup replacement frequency, measure milk conductivity during milking
3.Stalls and housing management	Outside access, type and frequency of alley cleaning, stall base, type, amount, and frequency of replacement of bedding in stalls, frequency of removing manure from stalls, part of the stall that is cleaned, use of a bacteriostatic product in stalls, technique used to disinfect stalls, cleanliness of cows' flanks, legs, and udder, stall dimensions, uses electric trainers, stocking density
4. Maternity pens	Proportion of calving occurring in a maternity pen (MP), number of MP, ratio MP per cow, use of individual *vs.* group MP, days spent in MP before and after calving, MP use for sick cows, MP base, type, amount, and frequency of replacement of bedding in MP, frequency of removing manure from MP, technique used to disinfect MP
5. General management and biosecurity	Fly control, udder hair management, tail management, vaccinates against coliform mastitis, general vaccination, records diseases, cow bought:milking cow ratio, tests udder health of purchased cows, routinely uses bacteriological analysis, routinely uses CMT, monitors individual production, reviews individual SCC frequently, has culling protocols for high SCC, *S. aureus*, and clinical mastitis cows, proportion of cows treated with antibiotics at dry-off

Table VIII. (Continued)

Manageable risk factors (continued)	
6. Nutrition	Manages calcium in diet around calving, feeding system, milking cows' diet balanced based on forage analysis, cow and herd milk protein/fat ratio
7. Management of clinical cases	Proportion of clinical mastitis treated, treatment duration, uses teat disinfection before intramammary (IMM) treatment, uses complete *vs.* partial insertion for IMM treatment, applies post-milking teat disinfection after IMM treatment
8. Demographics and infection prevalence	Herd and cow milk yield, herd quarter prevalence of *S. aureus,* CNS, *Corynebacterium* spp, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and other *Streptococci*
Covariates [1]	
9. Housing type, demographics, and infection prevalence	Type of housing system, season, mean number of milking cows, herd mean days in milk (DIM), cow parity and DIM, herd previous mean linear cell score (LCS), herd mean LCS for the preceding 24 months, cow previous LCS from DHI, cow number of DHI test with LCS > 4.0 in the last 3 tests, quarter LCS on 1st sample, cow and quarter prevalence of *S. aureus,* CNS, *Corynebacterium* spp, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and other *Streptococci*
10.Motivations and attitudes	Herd manager (HM) self-reported meticulousness, HM meticulousness (external evaluation), farm management level (external evaluation), HM enjoys milking (self-reported), record keeping completeness (external evaluation), HM wish to reduce bulk tank SCC (BTSCC), goal for BTSCC, importance of high SCC or *Staph. aureus* in culling decision, HM worries about cost of mastitis, udder health is important criteria for bull selection, HM thinks he should do more about mastitis prevention, resource persons used to prevent or solve mastitis problems, rationale for treatment decision of clinical cases
11. Knowledge and beliefs	Cow SCC and BTSCC threshold considered problematic, perceived ease of detecting high SCC cows, *S. aureus* perceived as an environmental problem, individual SCC measurements are important, perceived control over mastitis problems, self-evaluated knowledge on mastitis

[1] Variables listed as covariates were used only as potential confounders and/or effect modifiers of association between manageable risk factors and *Staphylococcus aureus* IMI.

Table IX (supplementary material)**.** Unconditional estimates of association between explanatory variables and odds of having an incident, eliminated, or prevalent *Staphylococcus aureus* IMI (variables with joint *P*-value ≤ 0.20).

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
1.Milking procedures						
Written milking procedures	0.75	0.51, 1.1			0.73	0.53, 1.0
Milkers receive bonus for milk quality	0.42	0.13, 1.4			0.42	0.16, 1.1
Milkers wear gloves	0.60	0.42, 0.86	1.9	1.1, 3.3	0.65	0.48, 0.87
Gloves cleaned during milking						
No cleaning			Ref	Ref		
Rinse with water			1.6	0.65, 3.7		
Clean with udder wash			0.74	0.18, 3.1		
Clean with iodine			0.26	0.07, 1.0		
Fore-strip prior to milking			1.4	0.86, 2.2		
Foremilk discarded						
In filter-cup			Ref	Ref		
On floor			2.3	1.2, 4.4		
In prepping towels			1.3	0.38, 4.7		
In gutter			0.92	0.50, 1.7		
Teats cleaned before milking			0.06	0.01, 1.5		
Teat cleaning method						
Dry-wipe	Ref	Ref			Ref	Ref
Pre-milking disinfection and wipe	0.70	0.34, 1.4			0.99	0.54, 1.8
Udder wash	1.1	0.54, 2.4			1.6	0.85, 3.0
Water	0.27	0.03, 2.8			0.52	0.11, 2.5
Commercial wet disinfecting towels	0.45	0.15, 1.3			0.60	0.25, 1.5

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
1.Milking procedures (continued)						
Teat drying method						
No drying			Ref	Ref		
Paper towels			0.57	0.25, 1.3		
Reusable cloth towels			1.1	0.42, 2.7		
Cloth towels used for > one cows during a milking	0.38	0.13, 1.1				
Uses pre-milking teat disinfection	0.63	0.43, 0.93	0.70	0.43, 1.1	0.67	0.50, 0.92
Sprayed post-milking teat disinfection (*vs.* dipped)					1.4	0.85, 2.2
Uses post milking teat disinfection			0.11	0.03, 0.51		
Milking order for high SCC cows						
No milking order					Ref	Ref
Milked last or with a specific unit					1.5	1.1, 2.1
Milk among other cows and unit is disinfected afterward					0.94	0.5, 1.7
≥ 4 milking units used per milkers	0.72	0.45, 1.2				
Uses technique to keep cows standing after milking						
No			Ref	Ref		
Fresh feed distribution			1.6	0.98, 2.6		
Fresh feed distribution and head locks			1.1	0.23, 5.0		
Direct cows toward feed alley			3.7	0.80, 17.4		

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
1.Milking procedures (continued)						
Teat end condition score [1]						
N	Ref	Ref	Ref	Ref	Ref	Ref
1A	0.88	0.55, 1.4	0.57	0.27, 1.2	1.0	0.74, 1.4
1B	0.93	0.57, 1.5	0.67	0.30, 1.5	0.93	0.66, 1.3
1C	1.1	0.63, 2.0	0.39	0.15, 1.0	1.2	0.77, 1.7
2A	1.1	0.67, 1.9	0.67	0.28, 1.6	1.0	0.69, 1.5
2B	1.2	0.74, 2.1	0.45	0.20, 1.0	1.3	0.93, 1.9
2C	1.9	0.97, 3.6	0.16	0.05, 0.55	1.6	0.99, 2.5
2D	3.1	1.5, 6.3	0.38	0.11, 1.3	3.0	1.77, 5.1
2.Milking equipment						
Vacuum level checked at least daily	1.5	0.98, 2.3			1.6	1.1, 2.2
Measures milk conductivity	2.1	1.0, 4.2	1.6	0.79, 3.4	2.0	1.1, 3.7
3.Stalls and housing management						
Outside access						
No outside access	Ref	Ref				
Access to exercise yard	0.29	0.10, 0.84				
Access to pasture	0.87	0.58, 1.3				
Type of stall base						
Mattress or rubber	Ref	Ref	Ref	Ref	Ref	Ref
Concrete	0.73	0.40, 1.3	0.68	0.28, 1.6	0.51	0.32, 0.81
Sand	0.37	0.18, 0.73	3.1	0.69, 13.8	0.31	0.19, 0.52

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
3.Stalls and housing management (continued)						
Type of bedding						
Wood products			Ref	Ref		
Sand			36.0	4.0, 325.7		
Straw			0.74	0.42, 1.3		
Hay			1.1	0.40, 3.2		
Wood and straw			0.92	0.41, 2.1		
Uses ≥ 2cm deep of bedding	0.72	0.49, 1.1			0.74	0.54, 1.0
Bedding adding frequency						
< once a day			Ref	Ref		
Once a day			0.47	0.23, 0.92		
> once a day			0.52	0.30, 0.89		
Technique used to disinfect stalls						
No cleaning/disinfection	Ref	Ref	Ref	Ref		
Disinfecting product	2.1	1.1, 4.3	0.90	0.42, 1.9		
Pressure wash only	1.1	0.60, 2.0	0.36	0.17, 0.77		
Stall length						
< 1.7 m	Ref	Ref			Ref	Ref
1.7-1.8 m	1.1	0.67, 2.0			1.1	0.76, 1.7
1.8-1.9 m	1.6	0.90, 3.0			1.5	0.95, 2.4
> 1.9 m	0.45	0.20, 1.0			0.45	0.24, 0.83
Stall width						
≤ 1.2 m	Ref	Ref	Ref	Ref	Ref	Ref
1.2-1.3 m	1.5	0.94, 2.4	0.47	0.26, 0.84	1.6	1.1, 2.3
> 1.3 m	1.8	1.0, 3.1	0.36	0.18, 0.72	1.8	1.2, 2.9

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
3.Stalls and housing management (continued)						
Stalls are equipped with a neckrail	0.60	0.33, 1.1				
Neck rail height						
< 1.1m			Ref	Ref		
1.1 – 1.2m			1.8	0.99, 3.3		
>1.2m			2.1	1.2, 3.7		
Distance neckrail-curb (m) [2]	1.1	0.98, 1.2				
Stocking density (freestall only)						
Ratio cows:stalls <105%			Ref	Ref	Ref	Ref
Ratio cows:stalls ≥105%			2.7	0.67, 11.1	0.54	0.26, 1.1
4.Maternity pens(MP)						
Number of MP						
0					Ref	Ref
1					0.72	0.49, 1.1
2					0.50	0.33, 0.76
3					0.49	0.30, 0.81
4					0.13	0.03, 0.51
5					0.16	0.04, 0.62
Cows left ≤ 1 day in MP after calving					0.63	0.40, 0.99
Type of bedding in MP						
Wood products					Ref	Ref
Straw					2.0	1.0, 3.8
Hay					7.0	1.5, 33.2
Wood products and straw					1.7	0.79, 3.7

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
4.Maternity pens(MP)(continued)						
Manure removed from MP:						
≥ once/day					Ref	Ref
< once/day and ≥ once/month					0.57	0.29, 1.1
< once/month					1.1	0.65, 1.7
After every calving					1.9	0.88, 3.9
As needed					1.5	0.68, 3.5
Technique used to disinfect MP						
No cleaning/disinfection			Ref	Ref		
Disinfecting product			0.90	0.35, 2.3		
Pressure wash only			0.39	0.17, 0.90		
5.General management and biosecurity						
Control flies			0.60	0.35, 1.0		
Ties, clips, or docks tails			1.6	0.80, 3.0		
Vaccinates against coliform mastitis			1.7	1.0, 2.7		
Purchase habits in preceding 6m						
Never buys cattle			Ref	Ref		
Usually buy cattle but not in last 6 mo			0.14	0.02, 0.90		
Purchased only heifers			0.41	0.18, 0.91		
Purchased cows			0.86	0.53, 1.4		
Purchased cows are:						
No purchased cows	Ref	Ref	Ref	Ref		
Not tested	0.65	0.30, 1.4	0.44	0.14, 1.4		
Checked for SCC or with CMT	0.75	0.47, 1.2	0.95	0.53, 1.7		
Checked with bacteriology	1.7	1.1, 2.8	0.56	0.32, 0.99		

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
5.General management and biosecurity (continued)						
Milk bacteriologic culture is:						
Not used routinely					Ref	Ref
Used on suspect cows					0.91	0.62, 1.3
Used on most cows once/y					1.4	0.96, 2.1
Culling protocol for high SCC cows						
No culling protocol	Ref	Ref			Ref	Ref
Culls if > 3 tests over chosen threshold	2.0	1.1, 3.7			1.8	1.1, 3.0
Culls if ≤ 3 tests over chosen threshold	0.95	0.50, 1.8			0.93	0.55, 1.6
Culling protocol for clinical mastitis (CM)						
No culling protocol			Ref	Ref	Ref	Ref
Culls cow if > 3 CM cases			0.35	0.15, 0.81	0.52	0.32, 0.84
Culls cow if 2 or 3 CM cases			0.99	0.56, 1.7	1.1	0.71, 1.6
6.Nutrition						
Manages calcium in diet at calving			1.5	0.94, 2.4		
Cow's milk protein:fat ratio						
≥ 0.75			Ref	Ref		
< 0.75 (subclinical ketosis)			2.0	1.2, 3.4		
7.Management of CM cases						
Treatment duration for CM cases > 2 days					0.73	0.53, 1.0
Applies post-milking teat disinfection after intramammary treatments			0.29	0.08, 1.1		

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
8.Infection prevalence						
Herd initial *Staphylococcus aureus* quarter prevalence [3]	1.7	1.5, 2.1				
9.Housing type, demographics and infection prevalence						
Housing type						
Tie-stall	Ref	Ref	Ref	Ref	Ref	Ref
Freestall	0.71	0.47, 1.1	1.7	1.1, 2.5	0.68	0.48, 0.91
Bedded pack barn	0.21	0.05, 0.80	0.76	0.06, 9.5	0.17	0.03, 0.86
>70 milking cows			1.6	1.1, 2.4		
Cow's parity						
First	Ref	Ref	Ref	Ref	Ref	Ref
Second	1.3	0.91, 1.7	0.46	0.26, 1.0	1.2	0.95, 1.5
Third	1.4	0.97, 2.0	0.66	0.35, 1.2	1.4	1.1, 1.7
> third	1.5	1.1, 2.1	0.56	0.30, 0.83	1.4	1.1, 1.8
Cow in early lactation (0-60 days)			2.0	1.2, 3.1		
Herd mean somatic cell score (SCS) in preceding 24 m	1.7	1.2, 2.4			---	---
Herd mean LCS in preceding 24 m						
≤ 2.0	---	---			Ref	Ref
2.0 – 3.0	---	---			1.1	0.60, 2.1
≥ 3.0	---	---			1.7	0.90, 3.3
Cow's previous DHI SCS [4]			0.77	0.71, 0.83	---	---
Cow's previous DHI SCS						
≤ 2.0			---	---	Ref	Ref
2.0 – 3.0			---	---	1.4	0.84, 2.4
3.0 – 4.0			---	---	3.7	2.2, 6.1
≥ 4.0			---	---	11.1	7.1, 17.3

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
9.Housing type, demographics and infection prevalence (continued)						
Cow's number of SCS > 4.0 in last 3 DHI tests						
0			Ref	Ref	Ref	Ref
1			0.41	0.26, 0.67	3.2	2.1, 4.9
2			0.30	0.18, 0.50	9.6	5.3, 17.1
3			0.22	0.13, 0.36	27.6	14.9, 51.1
Quarter's SCS on 1st sample [4]	1.4	1.3, 1.4	---	---		
Quarter's SCS on 1st sample						
< 4.0	---	---	Ref	Ref		
4.0-6.0	---	---	0.20	0.12, 0.35		
> 6.0	---	---	0.10	0.06, 0.17		
Cow's number of *S. aureus* infected quarters on 1st sample						
0	Ref	Ref	---	---		
1	4.5	3.3, 6.0	Ref	Ref		
2	6.8	3.8, 12.1	1.2	0.73, 2.0		
3	15.8	5.3, 46.8	0.85	0.36, 2.0		
4	---	---	0.05	0.01, 0.67		
Cow's number of CNS infected quarters on 1st sample						
0	Ref	Ref	Ref	Ref		
1	1.2	0.84, 1.6	1.2	0.74, 1.9		
2	0.89	0.63, 1.3	1.9	1.1, 3.2		
3	0.80	0.47, 1.4	1.3	0.58, 3.1		
4	1.5	1.0, 2.2	3.2	1.3, 7.4		

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
9.Housing type, demographics and infection prevalence (continued)						
Cow's number of other streptococci infected quarters on 1st sample						
0			Ref	Ref		
1			1.5	0.78, 2.9		
2			3.1	1.1, 9.2		
3			0.01	0.01, ∞		
4			10.6	1.2, 90.3		
CNS IMI in quarter on 1st sample	1.6	1.2, 2.1	3.6	1.8, 7.0	0.27	0.21, 0.34
Corynebacterium spp IMI in quarter on 1st sample			0.16	0.02, 1.4	0.34	0.23, 0.50
Streptococcus uberis IMI in quarter on 1st sample			7.1	0.38, 131		
Streptococcus dysgalactiae IMI in quarter on 1st sample	4.4	1.1, 18.1			11.8	4.6, 30.7
Prevalent other streptococci IMI in quarter on 1st sample					0.52	0.35, 0.76
10.Motivations and attitudes						
Herd manager's self-reported meticulousness (1-10 scale) [5]			1.2	1.0, 1.3[6]		
Importance of high SCC in culling decision						
Not important	Ref	Ref				
Neutral	0.50	0.22, 1.1				
Important	0.71	0.34, 1.5				
Importance of *S. aureus* status in culling decision						
Not important			Ref	Ref		
Neutral			0.38	0.19, 0.78		
Important			0.52	0.27, 0.98		

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
10.Motivations and attitudes						
Udder health is important in bull selection						
Disagree			Ref	Ref		
Neutral			0.95	0.50, 1.8		
Agree			1.9	1.1, 3.3		
I should do more about mastitis prevention						
Disagree	Ref	Ref				
Neutral	0.79	0.49, 1.3				
Agree	0.41	0.23, 0.73				
Veterinarian is consulted regarding mastitis problems						
Rarely					Ref	Ref
Sometimes					1.6	1.0, 2.6
Regularly					1.7	1.1, 2.6
Characteristics of the cow are important when deciding to treat a CM case:						
Not important			Ref	Ref		
Neutral			0.49	0.25, 0.94		
Important			0.68	0.41, 1.1		
Need for milk for quota is important when deciding to treat a clinical mastitis case						
Not important	Ref	Ref			Ref	Ref
Neutral	0.87	0.54, 1.4			0.77	0.52, 1.1
Important	0.56	0.34, 0.91			0.50	0.34, 0.74

Table IX. (Continued)

Explanatory variable	Incident IMI		Eliminated IMI		Prevalent IMI	
	OR	95% CI	OR	95% CI	OR	95% CI
10.Motivations and attitudes (continued)						
Cull cow price is important when deciding to treat a clinical mastitis case						
Not important	Ref	Ref				
Neutral	1.7	1.0, 2.8				
Important	0.75	0.34, 1.7				
Protocol established with veterinarian is important when deciding to treat a clinical mastitis case						
Not important					Ref	Ref
Neutral					0.67	0.44, 1.0
Important					0.67	0.46, 0.98
11.Knowledge and beliefs						
S. aureus is an environmental problem						
Disagree			Ref	Ref		
Neutral			1.9	1.1, 3.3		
Agree			1.1	0.57, 2.1		

[1] Teat end condition score as proposed by Neijenhuis et al. (2000).

[2] OR and 95% CI for a 10cm increase.

[3] OR and 95% CI for a 5% quarter prevalence difference.

[4] OR and 95% CI for one unit increase of SCS.

[5] OR and 95% CI for one unit increase of self-reported meticulousness on a 1 to 10 scale.

Table X. Conditional estimates of association between manageable risk factors and odds of acquisition of a new *Staphylococcus aureus* IMI estimated in Bayesian hierarchical cross-classified models. Only statistically significant manageable risk factors (95% CI not including the null value) are presented.

Modeled manageable risk factor	β	SE	OR	95% CI	Covariates [1]
Milkers wear gloves					Milkers receive bonus for milk quality, cow number of *S. aureus* infected quarters on 1st sample and its interaction term with gloves
Cow with no *S. aureus* quarters	-0.849	0.263	0.43	0.26, 0.72	
Cow with 1 *S. aureus* quarter	0.232	0.429	1.3	0.54, 2.9	
Cow with 2 *S. aureus* quarters	-1.447	0.788	0.24	0.05, 1.1	
Cow with 3 *S. aureus* quarters	-3.474	1.710	0.03	0.01, 0.89	
Uses pre-milking teat disinfection	-0.533	0.232	0.59	0.37, 0.92	---
Teat end condition score [2]					---
N	Ref	Ref	Ref [a]	Ref	
1A	-0.124	0.274	0.88[a,b]	0.52, 1.5	
1B	-0.049	0.287	0.95[a,b]	0.54, 1.7	
1C	0.161	0.342	1.2[a,b]	0.60, 2.3	
2A	0.151	0.308	1.2 [a,b]	0.64, 2.1	
2B	0.310	0.307	1.4 [a,b]	0.75, 2.5	
2C	0.754	0.386	2.1 [a,b]	1.0, 4.5	
2D	1.316	0.437	3.7[b]	1.6, 8.8	
Measures milk conductivity	1.286	0.479	3.6	1.4, 9.3	Housing type, herd mean SCS in preceding 24 m
Stall length					---
< 1.7 m	Ref	Ref	Ref [a]	Ref	
1.7-1.8 m	0.204	0.346	1.2 [a]	0.62, 2.4	
1.8-1.9 m	0.633	0.395	1.9 [a]	0.87, 4.1	
> 1.9 m	-1.009	0.531	0.36 [a]	0.13, 1.0	
Herd initial *S. aureus* quarter prevalence [3]	0.124	0.022	1.9	1.5, 2.3	---

[1] Covariates included in the model as confounders or effect modifiers to adjust the estimate of the manageable risk factor. Estimates of association are not reported for covariates, although they may have significantly differed from the null value.

[2] Teat end condition score as proposed by Neijenhuis et al. (2000).

[3] OR and 95% CI for a 5% quarter prevalence difference.

[a, b] For categorical variables with more than 2 classes, OR with different superscripts differ (adjusted for multiple comparisons using the Bonferroni procedure). All the presented risk factors were significantly associated with the outcome, but in some cases differences across levels of a risk factor could not be identified after correction for multiple comparisons.

Table XI. Conditional estimates of association between manageable risk factors and odds of elimination of an existing *Staphylococcus aureus* IMI estimated in Bayesian hierarchical cross-classified models. Only statistically significant manageable risk factors (95% CI not including the null value) are presented.

Modeled manageable risk factors	β	SE	OR	95% CI	Covariates [1]
Milkers wear gloves	0.803	0.372	2.2	1.1, 4.6	Use pre-milking teat disinfection, housing type
Stocking density (free-stall only)					Herd manager self-reported meticulousness, cow days in milk and its interaction term with stocking density
Cow 0-60 days in milk					
Ratio cows:stalls					
<105%	Ref	Ref	Ref	Ref	
≥105%	-1.583	1.663	0.21	0.01, 5.4	
Cow > 60 days in milk					
Ratio cows:stalls					
<105%	Ref	Ref	Ref	Ref	
≥105%	3.265	1.44	26.2	1.6, 440.3	
Culling protocol for clinical mastitis (CM)					Housing type, herd manager self-reported meticulousness, importance of udder health in bull selection, knowledge on *S. aureus* etiology
Culls cow if > 3 CM	Ref	Ref	Ref [a]	Ref	
No culling protocol	1.432	0.670	4.2 [a]	1.1, 15.6	
Culls cow if 2-3 CM	0.591	0.706	1.8 [a]	0.45, 7.2	

[1] Covariates included in the model as confounders or effect modifiers to adjust the estimate of the manageable risk factor. Estimates of association are not reported for covariates, although they may have significantly differed from the null value.

[a] For categorical variables with more than 2 classes, OR with different superscripts differ (adjusted for multiple comparisons using the Bonferroni procedure). All the presented risk factors were significantly associated with the outcome, but in some cases differences across levels of a risk factor could not be identified after correction for multiple comparisons.

Table XII. Conditional estimates of association between manageable risk factors and odds of a prevalent *Staphylococcus aureus* IMI estimated in Bayesian hierarchical cross-classified models. Only statistically significant manageable risk factors (95% CI not including the null value) are presented.

Manageable risk factors	β	SE	OR	95% CI	Covariates [1]
Milkers wear gloves	-0.470	0.200	0.63	0.42, 0.92	Housing type
Uses pre-milking teat disinfection	-0.460	0.206	0.63	0.42, 0.95	---
Teat end condition score [2]					
N	Ref	Ref	Ref[a]	Ref	---
1A	0.099	0.203	1.1[a,b]	0.74, 1.6	
1B	0.003	0.216	1.0[a,b]	0.66, 1.5	
1C	0.197	0.231	1.2[a,b]	0.77, 1.9	
2A	0.045	0.241	1.1[a,b]	0.65, 1.7	
2B	0.382	0.231	1.5[a,b]	0.93, 2.3	
2C	0.650	0.297	1.9[a,b]	1.1, 3.4	
2D	1.553	0.342	4.7[b]	2.4, 9.2	
Vacuum level checked at least daily	0.606	0.248	1.8	1.1, 3.0	---
Measures milk conductivity					Herd mean SCS in preceding 24 m, housing type and its interaction with conductivity
Tie-stall	-0.883	0.918	0.41	0.07, 2.5	
Free-stall	1.722	0.474	5.6	2.2, 14.2	
Bedded pack barn	2.457	1.386	11.7	0.77, 176.6	
Type of stall base					Housing type, cow parity and its interaction with type of stall base
1st parity cows					
Mattress or rubber	Ref	Ref	Ref[a]	Ref	
Concrete	-0.160	0.426	0.85[a]	0.37, 2.0	
Sand	0.102	0.525	1.1[a]	0.40, 3.1	
2nd parity cows					
Mattress or rubber	Ref	Ref	Ref[a]	Ref	
Concrete	-1.093	0.468	0.34[a,b]	0.13, 0.84	
Sand	-1.793	0.728	0.17[b]	0.04, 0.69	
3rd parity cows					
Mattress or rubber	Ref	Ref	Ref[a]	Ref	
Concrete	-2.929	0.962	0.05[b]	0.01, 0.35	
Sand	-1.269	0.679	0.28[a,b]	0.07, 1.1	
>3rd parity cows					
Mattress or rubber	Ref	Ref	Ref[a]	Ref	
Concrete	-0.542	0.518	0.58[a,b]	0.21, 1.6	
Sand	-1.833	0.748	0.16[b]	0.04, 0.69	

Table XII. (Continued)

Manageable risk factors	β	SE	OR	95% CI	Covariates [1]
Uses ≥2cm deep of bedding					Type of stall base, cow parity and its interaction with amount of bedding used
1st parity cows	0.467	0.286	1.6	0.91, 2.8	
2nd parity cows	-0.253	0.281	0.78	0.45, 1.3	
3rd parity cows	0.058	0.320	1.1	0.57, 2.0	
>3rd parity cows	-0.692	0.286	0.50	0.28, 0.91	
Stall length					Housing type, cow parity and its interaction with stall length
1st parity cows					
<1.7 m	Ref	Ref	Ref [a]	Ref	
1.7-1.8 m	-0.436	0.392	0.65[a]	0.30, 1.4	
1.8-1.9 m	-0.561	0.467	0.57[a]	0.23, 1.4	
>1.9 m	-1.097	0.578	0.33[a]	0.11, 1.0	
2nd parity cows					
<1.7 m	Ref	Ref	Ref [a]	Ref	
1.7-1.8 m	0.608	0.418	1.8 [a]	0.81, 4.2	
1.8-1.9 m	0.914	0.470	2.5 [a]	0.99, 6.3	
>1.9 m	-0.318	0.645	0.73[a]	0.21, 2.6	
3rd parity cows					
<1.7 m	Ref	Ref	Ref [a]	Ref	
1.7-1.8 m	0.367	0.464	1.4 [a]	0.58, 3.6	
1.8-1.9 m	1.035	0.502	2.8 [a]	1.1, 7.5	
>1.9 m	-0.860	0.716	0.42[a]	0.10, 1.7	
>3rd parity cows					
<1.7 m	Ref	Ref	Ref [a]	Ref	
1.7-1.8 m	0.370	0.440	1.4 [a]	0.61, 3.4	
1.8-1.9 m	1.203	0.506	3.3 [a]	1.2, 9.0	
>1.9 m	-2.020	0.880	0.13[a]	0.02, 0.74	
Number of maternity pens (MP)					Housing type
0	Ref	Ref	Ref [a]	Ref	
1	-0.365	0.271	0.69[a,b]	0.41, 1.2	
2	-0.797	0.303	0.45[a,b]	0.25, 0.82	
3	-0.761	0.342	0.47[a,b]	0.24, 0.91	
4	-2.570	0.926	0.08[b]	0.01, 0.47	
5	-2.218	0.906	0.11[a,b]	0.02, 0.64	
Culling protocol for clinical mastitis (CM)					---
Culls cow if > 3 CM	Ref	Ref	Ref [a]	Ref	
No culling protocol	0.890	0.351	2.4 [b]	1.2, 1.6	
Culls cow if 2-3 CM	1.003	0.424	2.7 [a,b]	1.2, 6.3	

Table XII. (Continued)

Manageable risk factors	β	SE	OR	95% CI	Covariates [1]
Treatment duration for CM cases > 2 days					Veterinarian consulted regarding mastitis problems and its interaction with treatment duration
Veterinarian consulted rarely	-1.094	0.490	0.33	0.13, 0.87	
Veterinarian consulted sometimes	-0.890	0.362	0.41	0.20, 0.83	
Veterinarian consulted regularly	0.423	0.336	1.5	0.79, 2.9	

[1] Covariates included in the model as confounders or effect modifiers to adjust the estimate of the manageable risk factor. Estimates of association are not reported for covariates, although they may have significantly differed from the null value.

[2] Teat end condition score as proposed by Neijenhuis et al. (2000).

[a, b] For categorical variables with more than 2 classes, OR with different superscripts differ (adjusted for multiple comparisons using the Bonferroni procedure). All the presented risk factors were significantly associated with the outcome, but in some cases differences across levels of a risk factor could not be identified after correction for multiple comparisons.

Figures

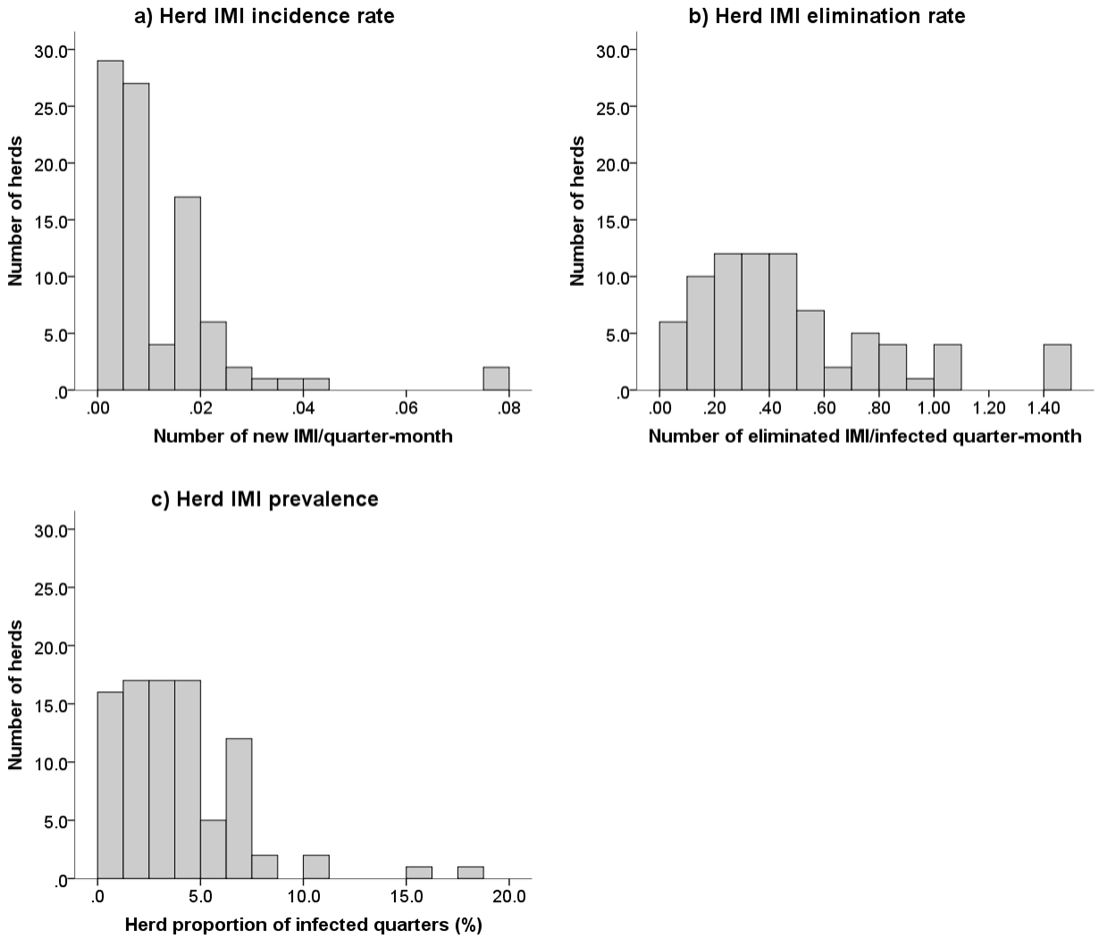

Figure 3. Herd *Staphylococcus aureus* IMI incidence rate, elimination rate, and prevalence distributions.

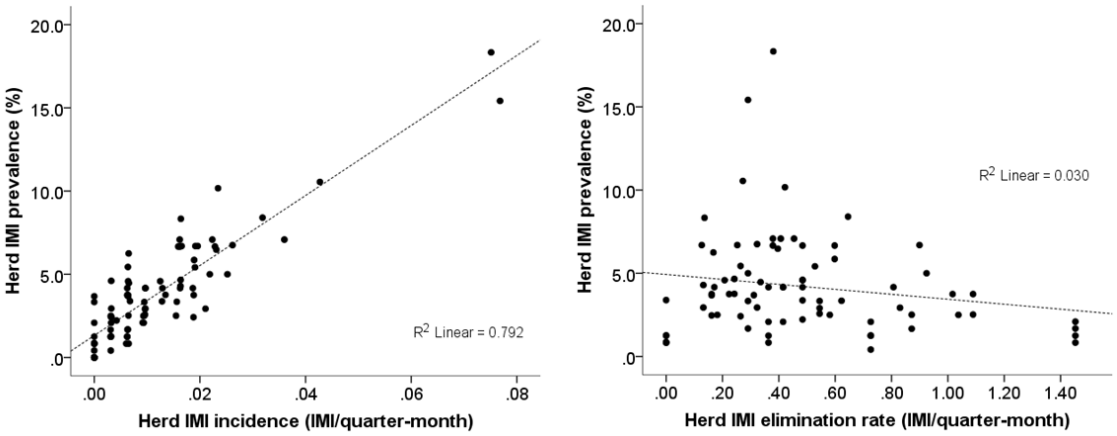

Figure 4. Scatter plots of herd *Staphylococcus aureus* IMI prevalence against herd IMI incidence and elimination rates.

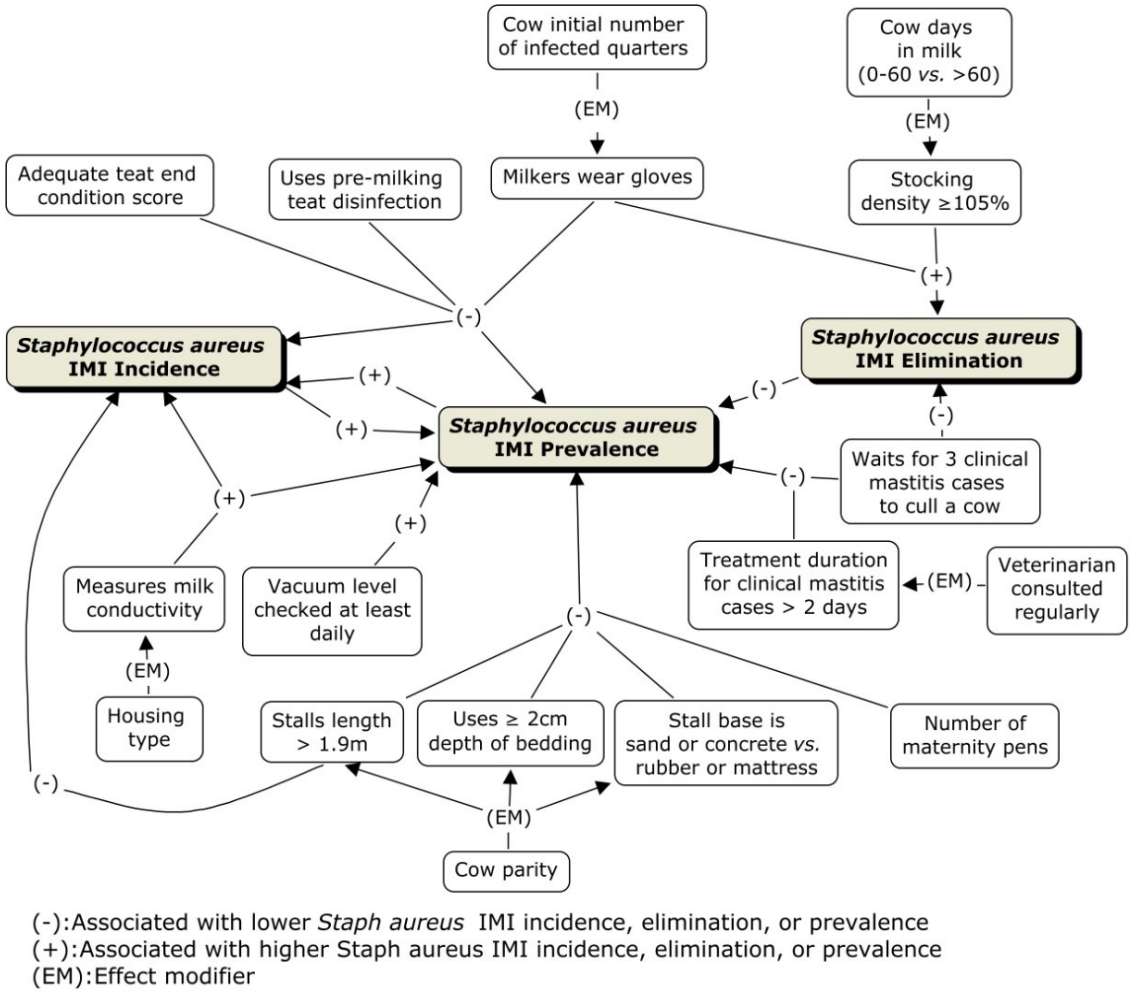

Figure 5. Conceptual chart of associations between manageable risk factors and *Staphylococcus aureus* IMI incidence, elimination, and prevalence.

Coagulase-negative staphylococci intramammary infection epidemiology in dairy cattle and impact of bacteriological culture misclassification

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Abstract

Objectives of this study were to identify the manageable risk factors associated with the lactational incidence, elimination, and prevalence of coagulase-negative staphylococci (**CNS)** intramammary infections (**IMI**) while taking into account the difficulties inherent to their diagnosis. A second objective was to evaluate the impact of CNS IMI misclassification in mastitis research. A cohort of 90 Canadian dairy herds was followed throughout 2007-2008. In each herd, series of quarter milk samples were collected from a sub-sample of cows and bacteriological culture was performed to identify prevalent, incident and eliminated CNS IMI. Practices used on farms were captured using direct observations and a validated questionnaire. The relationships between herd CNS IMI prevalence and herd incidence and elimination rates were explored using linear regression. Manageable risk factors associated with the prevalence, incidence, or elimination of CNS IMI were identified via Bayesian analyses using a latent class model approach allowing adjustment of the estimates for the imperfect sensitivity and specificity of bacteriological culture. After adjustment for the diagnostic test limitations, a mean CNS IMI quarter prevalence of 42.7 % (95% CI: 34.7, 50.1) and incidence and elimination rates of 0.29 new IMI/quarter-month (95% CI: 0.21, 0.37) and 0.79 eliminated IMI/quarter-month (95% CI: 0.66, 0.91), respectively, were observed. Considerable biases of the estimates were observed when CNS IMI misclassification was ignored. These biases were important for measures of association with risk factors, were nearly always toward the null value, and led to both Type I and Type II errors. Coagulase-negative staphylococci IMI incidence appeared to be a stronger determinant of herd IMI prevalence than IMI elimination rate. The majority of herds followed were already using blanket dry cow treatment and post-milking teat disinfection. A holistic approach considering associations with all 3 outcomes was employed to interpret associations between manageable risk factors and CNS IMI. Sand and wood-based product bedding showed desirable associations with CNS IMI compared to straw bedding. Quarters of cows that had access to pasture during the sampling period had lower odds of acquiring a new CNS IMI and of having a prevalent

CNS IMI. Many practices showed an association with only one of the CNS outcomes and should, therefore, be considered with caution.

Key words: dairy cow, mastitis, CNS, misclassification

Introduction

Historically, CNS IMI have received less attention compared to IMI caused by major pathogens such as *Staphylococcus aureus*, streptococci, and coliforms. One reason for this is that CNS IMI most often remain subclinical and generally lead to only mild to moderate SCC elevations compared to IMI caused by major mastitis pathogens (Djabri et al., 2002, Sampimon et al., 2010, Supré et al., 2011). With the gradually increasing control of IMI caused by major mastitis pathogens, however, recognition of the importance of CNS IMI and of their potential impact on udder health is rising. In recent studies conducted in different countries, CNS were the most common cause of IMI and were described as emerging mastitis pathogens (Pyörälä and Taponen, 2009, Sampimon et al., 2009a, Tenhagen et al., 2006). In a Dutch study, 10% of the quarters from low SCC cows and 15% of the quarters from high SCC cows had CNS cultured from their milk (Sampimon et al., 2009a). Similarly, in Germany, CNS was cultured from 8 to 11%, depending on parity and stage of lactation, of apparently healthy quarters (Tenhagen et al., 2006). Results from different studies are difficult to compare, though, since different definitions of what constitute a CNS IMI are often used. In addition, regardless of the CNS IMI definition used, the use of bacteriological culture to diagnose CNS IMI always produces a substantial level of IMI misclassification (Dohoo et al., 2011). In much research, misclassification bias is ignored or discussed strictly qualitatively. Nonetheless, relatively mild non-differential misclassification can yield, in some situations, a sizeable bias of the estimates of disease frequency and of association with exposures (Höfler, 2005).

Even though each CNS-infected quarter may only show a moderate increase in SCC, the often large proportion of infected quarters in a herd can still have an important impact on the bulk milk SCC (**BMSCC**). In a large field study in the USA, it was estimated that CNS IMI were responsible for 18% of the BMSCC in low BMSCC herds (<200,000 cells/ml), a BMSCC contribution substantially larger than those of any of the so-called major mastitis pathogens (Schukken et al., 2009). These results suggest that, in

herds where major mastitis pathogens have been controlled, CNS IMI are an important obstacle impeding further udder health improvement.

Although CNS IMI have been shown to have an impact on individual cow SCC and BMSCC, there is still much debate, however, on the harmful effect of acquiring a CNS IMI. In some studies cows or heifers with CNS IMI were shown to have a slightly higher daily milk production when compared to uninfected individuals (Compton et al., 2007; Piepers et al., 2010; Schukken et al., 2009). Milk production losses can be underestimated, however, when infected individuals are compared to healthy herd mates rather than to their own pre-infection milk production (Pyörälä and Taponen, 2009). It is plausible that higher producing cows or heifers would be more at risk of acquiring a CNS IMI than the other way around. In a study conducted by Matthews et al. (1990) CNS-infected quarters had lower odds of acquiring a *S. aureus* IMI than CNS-free quarters. In another study, however, an increase risk of *S. aureus* IMI acquisition was observed in CNS-infected quarters (Dufour et al., In press). It is still unclear whether or not there is a real protective effect of CNS IMI against *S. aureus* IMI. It is also unclear whether a hypothetical beneficial effect resulting from a few potentially averted *S.aureus* IMI would compensate for a higher CNS prevalence. With the available knowledge on CNS IMI, preventing these IMI seems to remain an appropriate recommendation.

Preventing new CNS IMI is the key determinant for long-term reduction and control of these IMI. Little is known, however, about effective strategies for CNS IMI prevention. A recent study has investigated risk factors associated with CNS IMI prevalence in early lactation of dairy heifers (Piepers et al., 2011), while another examined the risk factors associated with CNS IMI herd prevalence (Sampimon et al., 2009b). No studies could be found in the literature to have been conducted on risk factors associated with the acquisition or the elimination of CNS IMI during the lactation.

The study presented is a longitudinal cohort study on acquisition and elimination of CNS IMI during lactation on 90 Canadian dairy herds. The main objective was to identify manageable risk factors associated with the incidence, elimination, and prevalence of these

IMI while taking into consideration the difficulties inherent to the diagnosis of CNS IMI. A secondary objective was to evaluate the impact of CNS IMI misclassification on estimates of disease frequency and on estimates of association with risk factors.

Materials and methods

The herds selected were members of the National Cohort of Dairy Farms (**NCDF**) of the Canadian Bovine Mastitis Research Network (**CBMRN**). A complete description of the herd selection process as well as of the characteristics of these herds has been published previously (Reyher et al., 2011). Briefly, 91 herds were recruited in 4 regions of Canada to participate in a 2 yr (2007 and 2008) cohort study. Early in 2007, one herd refused to pursue participation because of the extent of work involved. The study presented in this manuscript was carried out with data from the 90 herds that participated to the NCDF for at least one yr.

During the 2 yr course of the study, management practices in place and other important farm conditions were measured on multiple occasions using direct observations and a validated questionnaire (Dufour et al., 2010). These repeated observations were designed to allow the use, in subsequent analyses, of the practices and conditions in place at the beginning of each of 4 different sampling periods rather than merely those employed at the beginning of the cohort study. Management practices under investigation have been described thoroughly elsewhere (Dufour et al., 2010, Dufour et al., 2011a) and could be summarized in 8 categories: 1) milking procedures; 2) milking equipment; 3) stalls and housing; 4) maternity pens; 5) general management and biosecurity; 6) nutrition; 7) clinical mastitis; and 8) demographic and IMI prevalence. Attitudes, motivations, knowledge, and beliefs of dairy producers were also investigated as on-farm conditions that could potentially modify the effect of the practices under investigation. Individual and herd level milk production and SCS data, as well as herd demographic data were obtained from Dairy herd improvement records from 2005 to 2009. A complete description of the data

collection process as well as the prevalence of use of the selected management practices on the NCDF herds can be found in Dufour et al. (2010).

Milk Sampling

At the beginning of each of 4 different sampling periods (March-May 2007, June-August 2007, January-March 2008, and June-August 2008), a sample of 15 apparently healthy lactating cows from each NCDF herd was selected. During each sampling period, series of 3 milk samples were collected from each quarter of the selected cows at intervals of 3 wks by a team of trained technicians using a standardized protocol (Reyher et al., 2011). Signs of inflammation of the quarter and teat end condition scores (Neijenhuis et al., 2000) were recorded. Quarters showing signs of clinical mastitis were excluded. Cows that were treated for conditions other than mastitis were not excluded. Bacteriological culture of the milk samples was carried out using a protocol based on NMC guidelines (Hogan et al., 1999). Ten µl of milk was streaked on a Columbia agar +5% sheep blood plate and incubated aerobically at 35ºC for 24h. The different types of colonies were enumerated (up to 10 colonies) and speciated after 24h using recommended bacteriologic procedures, then re-incubated for another 24h. SCC measurements were obtained for each quarter milk sample using the Fossomatic milk cell counter (Fossomatic 4000 series, Foss Electric, Hillerød, Denmark).

Quarter milk samples for which 3 or more pathogen species were cultured were considered contaminated and were excluded. A quarter was considered infected with CNS whenever bacteriological culture yielded ≥ 100 phenotypically identical CNS cfu/ml of milk. This threshold was chosen based on the results from Dohoo et al. (2011). The same threshold was chosen to define IMI due to *S. aureus*, *Corynebacterium* spp, *Streptococcus uberis*, *Streptococcus agalactiae, Streptococcus dysgalactiae*, and other streptococci species (presumably, primarily enterococci). Pathogen-specific quarter, cow, and herd prevalence of IMI at the first sampling of each sampling period were computed for the previously mentioned pathogens and investigated as explanatory variables.

For each outcome (incidence, elimination, and prevalence of CNS IMI) a different dataset was constituted. To investigate CNS IMI incidence and elimination, samples from each series were organized in 2 pairs (1st and 2nd samples, 2nd and 3rd samples) and pairs with incomplete results were discarded (i.e. pairs with a contaminated sample). Only pairs negative for CNS on the 1st sample of the pair were considered at risk of becoming infected and an incident IMI was deemed to have occurred if CNS was cultured from the following sample. Conversely, only pairs where CNS was cultured from the 1st sample of the pair were considered at risk of eliminating an existing CNS IMI which was deemed to have occurred if the following sample was negative. Based on these definitions, outcomes for the incidence and elimination datasets were, respectively, acquisition and elimination of a CNS IMI over a 3-week period (i.e. between milk samples of a pair).

For CNS IMI prevalence, the series of quarter milk samples collected during a specific sampling period were considered as one single observation. A prevalent CNS IMI was deemed to be present if 1 or more of the 3 samples collected was found to be positive for CNS. Series where CNS was never cultured were defined as free of CNS IMI. The outcome for the prevalence data set was, therefore, the presence of a CNS IMI in any of the milk samples of a series. Based on these definitions, 3 separate data sets specific to each of the 3 outcomes of interest (CNS IMI incidence, elimination, and prevalence) were generated.

Analyses

First, the 2 yr CNS IMI incidence rate, elimination rate, and prevalence were computed for each NCDF herd. Next, the relative impact of incidence and elimination rates on the prevalence of CNS IMI was investigated using a linear regression model with dependent variable (the computed 2 yrs CNS IMI herd prevalence) and explanatory variables (the herd incidence and elimination rates).

Screening of Explanatory Variables

Descriptive analyses were conducted for each variable in each of the 3 datasets to identify distributions and unlikely values. In one herd, pre-milking teat disinfection and wearing gloves during milking were only used by half of the milkers; observations from this herd for these specific variables were, therefore, excluded from subsequent analyses. Only one of the participating herds had not implemented post-milking teat disinfection (**PMTD**). This practice was, therefore, not retained as an explanatory variable since its measure of association would be perfectly confounded by other characteristics specific to this herd. Finally, back-flush of the milking units between groups of cows was also used in one herd only and was not retained as explanatory variable for the same reason. Finally, maternity pen variables were not considered in the incidence analyses since cows were not exposed to these variables anymore when CNS IMI acquisition was measured during the lactation.

Next, for each outcome (acquisition of a CNS IMI over a 3-week period, elimination of an existing CNS IMI over a 3-week period, and presence of a CNS IMI in a series of milk samples), unconditional associations between explanatory variables and occurrence of the outcome were estimated. Explanatory variables at the herd, cow, quarter, and pair (of samples) level were considered. The correlation structure of the data was a hierarchical cross-classified structure. Briefly, although 2 pairs of observations were available per quarter during a sampling period, the definitions used for incident and eliminated IMI precluded correlation of observations per quarter per sampling period. For instance, a quarter acquiring an IMI on the first pair (first sample of the pair negative, second sample positive) would not be considered at risk of acquiring a new IMI for the second pair (first sample of the pair is positive), thus pairs of samples collected on a quarter during a sampling period could be considered independent observations. In the prevalence dataset, only one observation was considered per quarter during a sampling period, therefore precluding any quarter correlation within a sampling period. For the 3 outcomes, however, observations were clustered within cow, and, since cows could be randomly selected in multiple sampling periods, observations from some cows could be cross-

classified by herd and by sampling period. In all 3 datasets, however, most of the cows were randomly selected for only one sampling period, and only 18%, 2%, and < 1% of cows were selected for respectively 2, 3, and all 4 sampling periods. To facilitate the first stages of the analyses, unconditional analyses were carried out using a hierarchical logistic regression model which accounted only for cow and herd clustering of observations. These analyses were performed with the GLIMMIX procedure of SAS 9.2 (SAS Institute Inc., Cary, NC) using Laplace approximation. For continuous variables, linearity was evaluated by visual inspection of the lowess smoothed curve of the relationship between the continuous variable and the log odds of the outcome (Dohoo et al., 2009); variables were categorized whenever the linearity assumption could not be met. Variables with $P \leq 0.20$ (Wald test) were retained as potentially important explanatory variables. Pearson and Spearman correlation coefficients were computed among the retained variables to identify co-linearity issues ($\rho < -0.6$ or $\rho > 0.6$).

Rough Models Construction

For each outcome, a putative causal diagram based on theoretical background was developed with the retained variables to identify potentially important confounders and effect modifiers. A stepwise backward selection strategy was then used to construct a rough model for each of the 3 outcomes using the previously described simplified logistic hierarchical model. In these models, only the retained variables that could theoretically be modified relatively easily (referred to as "manageable risk factors" in the remainder of the manuscript) were tested for inclusion. Initial quarter, cow, or herd prevalence of IMI by pathogens other than CNS were strictly considered in these models as potential confounders or effect modifiers. Initial quarter SCC measurements were treated likewise. A relatively liberal *P* value of 0.10 was chosen as the inclusion criterion so variables that might have been significantly associated with the true outcome (the true unmeasured CNS IMI status) would not be excluded because of the inability to correctly and precisely measure this outcome using routine bacteriological culture. During the selection process, variables identified as potential confounders in the putative causal diagram were included in the

model whenever one of the confounded variables was present. For each management practices included in the model, a maximum of three logically-plausible effect modifiers were then tested. These effect modifiers were included in the model if a Wald test conducted on the cross-product terms yielded a *P* value lower than 0.05/n, where n was the total number of effect modifiers tested in the model (Bonferroni adjustment for multiple comparisons).

Misclassification Adjustment of the Models

Estimates from these 3 rough models were then revised to take into account the cross-classified part of the structure and to correct for the likely CNS IMI status misclassification due to the imperfect sensitivity (**Se**) and specificity (**Sp**) of bacteriological culture. For this last step, a Bayesian approach using a latent class model similar to the one described by McInturff et al. (2004) was used. A latent class model relates an observed variable to a latent unmeasured variable; in this study the IMI status measured using milk bacteriological culture needed to be related to the true but unmeasured quarter IMI status. With the proposed approach, prior distributions for the Se and Sp of the test used to measure the outcome can be used to relate the latent and observed variables. In this study, estimates of Se and Sp of bacteriological culture obtained using NCDF bacteriological isolates (Dohoo et al., 2011) were used to generate prior distributions for CNS IMI misclassification parameters. In this latent class model, misclassification of IMI status was deemed to be independent of the others variables in the model (non-differential misclassification). For instance, misclassification of the CNS IMI status of a quarter milk sample was deemed to be independent of the management practices used on the farms.

The impact of misclassification of exposures has been well described by Gustafson (2004) and, in some situations, will also lead to an important and sometimes unpredictable bias of the estimate of association and of its standard error. A validation study was, therefore, conducted with the NCDF participants to obtain Se and Sp estimates of the exposure measurements obtained using a questionnaire compared to direct observations (Dufour et al., 2010). For some exposures that could not directly be observed, estimates of

repeatability rather than Se and Sp were available; in this situation the method proposed by Lash et al. (2007) was used to generate Se and Sp estimates. Sensitivity and Sp estimates of the explanatory variables were inspected, and these variables were further categorized when needed in order to restrict the magnitude of the potential misclassification bias. This bias was minimized by ensuring that moderately observed exposures (30-70%) used in the analyses had both Se and Sp estimates ≥ 0.90, that uncommonly observed exposures (≤30%) had Sp estimate ≥ 0.95, and, finally, that commonly observed exposures (≥70%) had Se estimates ≥ 0.95. These values were chosen based on results from Höfler (2005) to restrict the analyses to situations where the magnitude of exposure misclassification bias was likely to be small.

During this last phase of analyses, the complete cross-classified hierarchical structure of the data was taken into account. The informative prior distributions specified for the misclassification parameters (Se and Sp) are described in Table XIII. Briefly, uni-modal beta distributions centered on the Se and Sp estimates obtained using NCDF isolates and reported in Dohoo et al. (2011) were chosen for Se and Sp. Furthermore, these distributions were truncated at values of more and less than 5 percentage-points around the reported estimate. This latter restriction was implemented to avoid less probable and sometimes inappropriate Se and Sp combinations and, therefore, improve convergence of the Markov chain Monte Carlo (**MCMC**) chains. In addition, for the IMI prevalence analysis, different Se and Sp prior distributions were used for series where one (n=1,439), 2 (n=4,852), or 3 (n=13,551) culture results were available to account for the increasing Se and decreasing Sp resulting from the parallel interpretation of multiple diagnostic tests (Dohoo et al., 2009). Non-informative prior distributions were used for the risk factors and random effects parameters. To evaluate the impact of using traditional analyses where IMI misclassification is usually ignored, the 3 models were also run with Se and Sp of exactly 100%.

Finally, traditional and misclassification-adjusted estimates of the mean CNS IMI prevalence and incidence and elimination rates were obtained using the same approach. To

achieve this, models with only an intercept (β_0) and random effects was used for each outcome using first Se and Sp estimates of exactly 100% and then the Se and Sp estimates presented in Table XIII. Mean estimates of prevalence, incidence rate, and elimination rate were then obtained by transformation of their respective intercepts using the following formula (Dohoo et al., 2009):

_____ [1]

Incidence and elimination rates were then converted to number of events per quarter-month.

Inferences presented were obtained using WinBUGS 1.4.3 (MRC Biostatistics Unit, Cambridge, UK). These were based on MCMC samples of size 75,000 composed of 3 different chains with different starting values. Visual inspection of the trajectories and of the evolution of the Gelman-Rubin statistic were used to monitor the convergence of the chains (Ntzoufras, 2009). Plots of the chains autocorrelation were inspected and thinning of the chains was used when appropriate. The WinBUGS code used is dysplayed in Annexe III. There were no further attempts to prune off the models from the variables that were not statistically significant after the misclassification adjustment was conducted. In the revised models, explanatory variables with 95% credibility interval not containing the null value (1.0) were considered statistically significant.

Results

Herds selected in this study milked on average 85 cows (range 32 to 326) and had a mean 305-d milk production of 9,781 kg of milk (range 7,734 to 12,377). A complete description of the NCDF herds can be found in Reyher et al. (2011). Over the 2 yr course of the study, 59,167 quarter milk samples were collected; 67 samples were lost or damaged before bacteriological culture could be realized, 159 samples were excluded because signs

of clinical mastitis (mastitis score > 0) were observed, and 7,145 samples were excluded because 3 or more pathogen species were cultured.

The non-adjusted herd CNS IMI quarter prevalence and incidence and elimination rates were all normally distributed with respective medians (25^{th} and 75^{th} percentiles) of 58.8% (47.2, 67.3), 0.36 new CNS IMI/quarter-month (0.28, 0.49), and 0.76 eliminated CNS IMI/quarter-month (0.67, 0.86). Both herd CNS IMI incidence and elimination rates were significant ($P \leq 0.05$) predictors of the herd prevalence. Scatter plots of the relationships between herd prevalence and incidence and elimination rates are displayed in Figure 6. The herd incidence rate had a greater impact on the herd prevalence than the elimination rate. An increase of the herd incidence rate by its inter-quartile range (0.21 new IMI/quarter-month) was associated with an increase of the herd prevalence of 16.5 percentage-points. An equivalent decrease of the herd elimination rate by its inter-quartile range (0.19 eliminated IMI/infected quarter-month) was associated with an increase in the herd prevalence of only 2.3 percentage-points.

Risk Factors

CNS IMI Incidence

The incidence data set was composed of 20,354 pairs of milk samples at risk of becoming infected. These pairs were obtained from 11,221 quarters belonging to 3,707 cows. A new CNS IMI was identified in 5,009 of these pairs. When correcting for misclassification due to imperfect Se and Sp of bacteriological culture, a CNS IMI incidence of 0.29 new IMI/quarter-month (95% CI: 0.21, 0.37) was observed. In comparison, an incidence rate of 0.36 new IMI/quarter-month (95% CI: 0.32, 0.40) was estimated when misclassification was ignored. The direct consequences from the imperfect Se and Sp of bacteriological culture coupled with the observed prevalence of CNS IMI were, therefore, a substantial overestimation of the true CNS IMI incidence rate and an overly narrow confidence interval.

Conditional estimates of associations between manageable risk factors and odds of CNS IMI acquisition are presented in Table XIV. Quarters of cows that had access to pasture during the sampling period had lower odds of acquiring a new CNS IMI compared to quarters of cows that were confined inside. The type of bedding used in lactating cows' stalls or pens was significantly associated with CNS IMI acquisition; use of sand or wood-based product bedding was associated with lower odds of acquiring a CNS IMI compared to straw bedding. Lower odds of CNS acquisition were observed in herds where milkers received a bonus for milk quality.

For the incidence risk factors analysis, ignoring CNS IMI misclassification resulted in a bias toward the null value for all of the computed measures of association. In addition, IMI misclassification lead to narrower interval estimates for these measures. For this analysis, however, ignoring IMI misclassification was not resulting in any Type I (association wrongfully identified as statistically significant) or Type II (association wrongfully identified as insignificant) errors.

CNS IMI Elimination

The elimination dataset comprised 10,054 pairs of milk samples at risk of eliminating a CNS IMI. These pairs of samples were obtained from 7,132 different quarters from 3,304 cows. An elimination of the existing CNS IMI was observed in 5,121 of these pairs. When correcting for imperfect Se and Sp of the bacteriological culture, an estimate of 0.79 eliminated IMI/infected quarter-month (95% CI: 0.66, 0.91) was observed. When misclassification was ignored, an estimate of 0.80 eliminated IMI/infected quarter-month (95% CI: 0.75, 0.86) was obtained. Coagulase-negative staphylococci IMI elimination rate was therefore only slightly overestimated when IMI misclassification was present. The width of the associated 95% confidence interval was, however, grossly underestimated.

Results from the final model on risk factors associated with CNS IMI elimination are reported in Table XV. Briefly, the use of sand bedding was associated with higher odds

of IMI elimination. Higher odds of IMI elimination was also seen for quarters of cows with very dirty lower leg. Lower odds of CNS IMI elimination were seen when straw was used as bedding in maternity pens and when new bedding was added fewer than one time per day in these pens. Lower odds of IMI elimination was also seen in herds where milk conductivity was measured during milking. Finally, higher odds of CNS IMI elimination was seen in herds where cows have been purchased in the preceding 6 mo.

Like the incidence analysis, ignoring misclassification lead to bias of the odds ratio toward the null value and to narrower confidence intervals. In addition, a Type II error was made (lower leg cleanliness score) when CNS IMI misclassification was ignored.

CNS IMI Prevalence

The prevalence dataset contained 19,842 series of quarter milk samples. These series of samples were obtained from 15,771 different quarters from 3,998 cows. A total of 11,603 CNS-positive series were observed. Of these, 7,054 series (60.8%) had one CNS-positive sample, 3,183 (27.4%) had 2 positive samples, and for 1,366 series (11.8%), all 3 samples were positive for CNS. After adjusting for IMI misclassification, the true CNS IMI prevalence was estimated to be 42.7% (95% CI: 34.7, 50.1%). When IMI misclassification was ignored, a prevalence of 60.8% (95% CI: 57.1, 64.1%) was estimated. Ignoring IMI misclassification, therefore, resulted in a gross overestimation of the true CNS IMI prevalence and, again, in a too narrow 95% confidence interval.

Results from the model on the manageable risk factors for CNS IMI prevalence are reported in Table XVI. Similar to the incidence model, quarters of cows that had access to pasture during the sampling period had lower odds of having a prevalent IMI. In herds using sand or wood-based product bedding, a lower CNS IMI prevalence was observed. Odds of having a CNS IMI generally increased, although non-significantly, with the initial average herd SCS. This increase was constant across bedding type with the exception of hay bedding, for which a significant and steep decrease of the odds of a CNS IMI was seen with increasing average herd SCS. Lower odds of a prevalent IMI were seen in herds

where cows were left in a maternity pen for more than a week following calving. Finally, providing a bonus to milkers for milk quality and drying teats with paper or cloth towels as part of the milking procedures were associated with lower CNS IMI prevalence.

For the prevalence analysis, ignoring misclassification resulted, for nearly all measures of association, in a bias toward the null value. For one estimate (sand bedding and herd SCS interaction term; a continuous variable), however, a bias away from the null value was observed. All confidence intervals were narrower when misclassification was ignored and one Type I (feed total mixed ration) and one Type II (milkers receive bonus for milk quality) errors were made.

Discussion

This is the first longitudinal study reporting lactational incidence and elimination rates of CNS IMI and the manageable risk factors associated with acquisition and elimination of these in a large sample of herds over an extended period of time. An important strength of this study was the attempt to account for the imperfect Se and Sp of bacteriological culture for identifying CNS IMI. There is still a lack of agreement in the scientific community on what constitutes a CNS IMI, and efforts should therefore be made to link the milk bacteriological culture results interpreted within a given IMI definition to the proper quarter IMI status. Using the method proposed by McInturff et al. (2004) or simpler methods developed for 2x2 tables (Lash et al., 2009) would certainly improve the comparability across studies. In this study, for instance, CNS IMI was identified in 42.7% of apparently healthy mammary quarters. In comparison, a quarter prevalence of 42% was observed in early lactating heifers in Belgium (Piepers et al., 2011) but using a CNS IMI definition requiring ≥ 200 CNS cfu/ml of milk. In Germany (Tenhagen et al., 2006) and in the Netherlands (Sampimon et al., 2009a), using IMI definitions of ≥ 1,000 and ≥ 500 CNS cfu/ml of milk respectively, quarter prevalences of 8 to 11% and 10 to 15% were reported.

It is difficult indeed to directly compare these results because of the different IMI definitions used and the lack of adjustment for these imperfect definitions.

In this study, a CNS IMI definition of ≥ 100 phenotypically identical CNS cfu/ml of milk was used. This less specific but more sensitive definition was chosen to optimize the negative predictive value (**NPV**) of the diagnostic test used to diagnose the outcome, but also to initially select quarter at risk of becoming infected. Essentially, a less sensitive IMI definition would have lead to the incorrect inclusion of a larger number of already infected quarters in the incidence analysis, which was deemed to be the most important part of this study. For instance, assuming a prevalence of CNS IMI of 40%, and using the Se and Sp estimates reported in Dohoo et al. (2011), when requiring ≥ 200 CNS cfu/ml of milk, 24% of the recruited quarters would actually be already infected and, thus, wrongly recruited (NPV: 76%). This proportion would be reduced to 13% (NPV: 87%) with a ≥ 100 CNS cfu/ml of milk IMI definition. The ≥ 100 CNS cfu/ml IMI definition was, therefore, chosen to reduce a selection bias that could not be handled analytically. Under the same assumptions, using the ≥ 100 CNS cfu/ml of milk IMI definition to diagnose subsequent acquisition of a new CNS IMI would, however, result in a higher, but not as spectacular, proportion of wrongly identified new IMI (20%), when compared to the ≥ 200 CNS cfu/ml IMI definition (12%). This potentially greater misclassification bias could, however, be handled analytically with the latent class model used to adjust estimates of disease frequency and of association with exposures. In fact, when using such analytical treatment of misclassification bias, the choice of a specific IMI definition over another should not significantly alter the results, as long as well informed Se and Sp distributions can be specified for the chosen definition. To illustrate this point, the presented incidence model was also ran using a ≥ 200 CNS cfu/ml IMI definition to diagnosed acquisition of a new CNS IMI, and using a similar latent class model with Se and Sp distributions centered on 0.56 and 0.95, respectively (the Se and Sp estimates for a ≥ 200 CNS cfu/ml IMI definition reported in Dohoo et al., 2011). When comparing measures of association between the 2 misclassification-adjusted models, measures of association obtained using the ≥ 100 cfu/ml misclassification-adjusted model corresponded, on average, to 95% of those obtained using

the ≥ 200 cfu/ml IMI misclassification-adjusted model (data not shown). Using the ≥ 100 cfu/ml CNS IMI definition, therefore, resulted in only very slightly weaker measures of association with exposures and should not impact the results from these analyses.

Impact of CNS IMI Misclassification

In this study, ignoring CNS IMI misclassification yielded substantial bias of most measures of disease frequency. Usually, investigators tend to rely on intuition to qualitatively discuss how the misclassification bias may affect their results. In the authors' opinion, relying solely on intuition is unlikely to lead to a correct appraisal of the magnitude and direction of the resulting biases. Even for relatively simple analyses, such as estimating IMI prevalence, the resulting bias will be influenced by 3 components: the frequency of the disease in the population; the test Se; and the test Sp. While the bias can very easily be assessed quantitatively, correctly appraising the combined impacts of these 3 components qualitatively is very difficult. As observed by Lash (2007), when asked to intuitively appraise such bias, the vast majority usually fail to take into account the frequency of the disease in the population. In this study, most would have wrongfully guessed, for instance, that the relatively low test Se for CNS IMI would result in an underestimation of the true CNS incidence.

Important biases were also seen on measures of association with manageable risk factors. In the incidence and prevalence models, for instance, traditional regression coefficients corresponded, in general, to roughly 50% of the misclassification adjusted coefficients (Table XIV and XVI). In the elimination model, they corresponded more or less to 30% of the misclassification adjusted ones (Table XV). Although bias away from the null value was seen, the resulting biases were nearly always toward the null value, as would be expected with non-differential misclassification of binary variables. At first sight, Type I errors may seem nearly impossible with a bias toward the null value, but it is not once the grossly underestimated 95% confidence intervals are taken into consideration. Therefore, although direction of the biases was often predictable, these biases were sufficient to lead to either type I or type II errors. In this study, pretending that the outcome

was measured perfectly would have lead to different recommendations to dairy producers. Similar findings have been reported before by McGlothlin et al. (2008) and by Tarafder et al. (2011). Finally, estimates of association with exposures reported in the literature are commonly used latter on in economic studies, meta-analyses, or for the computation of other epidemiologic measures such as population attributable fractions. Reporting unadjusted estimates in one scientific manuscript is, therefore, very likely to lead to a certain number of subsequent erroneous recommendations.

Results from this study clearly highlight the important impact of ignoring CNS IMI misclassification. The method proposed by McInturff et al. (2004), however, can be used to handle this problem and offers many particularities that make it extremely interesting for mastitis research: it can correctly estimate both measures of disease frequency and measures of association with exposures; it can easily deal with hierarchical data structure; and, finally, uncertainty around Se and Sp estimates can be built-in.

CNS Epidemiology

As for many diseases, the rate at which new CNS IMI were acquired seemed to be a stronger determinant of the herd IMI prevalence than the elimination rate. These results would suggest that the control of risk factors associated with CNS IMI incidence would have a greater impact over time than the control of risk factors associated with elimination of existing CNS IMI. Actually, the relatively high CNS IMI incidence rate is certainly a striking feature of CNS IMI epidemiology compared to other common mastitis pathogens. Assuming that CNS IMI acquisitions are evenly distributed across quarters, a healthy quarter would have 29% chance of getting infected in any one month period which translates into 87% chance of getting infected over a 6 month period. On the NCDF farms, CNS IMI yielded by far the highest incidence rate among the mastitis pathogens reported (S. Dufour, unpublished data). In contrast, *S. aureus* incidence rates of 0.012 (Dufour et al., 2011a) and 0.019 new IMI/quarter-month (Zadoks et al., 2001) have been reported. With the often short duration (Supré et al., 2011, Taponen et al., 2007) and high prevalence of infection reported for CNS, that CNS would have such a high IMI incidence rate was

already suspected, and these results only corroborate this general belief. Coagulase-negative staphylococci IMI natural elimination rates have been reported before (Deluyker et al., 2005, McDougall, 1998, Taponen et al., 2006) and were quite variable across the populations studied and across the IMI definitions used. Although it cannot be directly compared to previously published studies, the CNS IMI elimination rate of 0.79 eliminated IMI/infected quarter-month observed in this study would be considered rather high. This high elimination rate could be the result of specific differences on Canadian farms in either or both the CNS species found and the host characteristics altering the response to these IMI. In a convenient sample of 387 of the NCDF CNS isolates recovered from apparently normal milking cows and speciated using gene sequencing, a large proportion (49.4%) were found to be *Staphylococcus chromogenes* (J.R. Middleton, unpublished data). In term of most frequent CNS species, therefore, the CNS isolates in this study would be comparable to those of studies conducted in the U.S. (Gillespie et al., 2009), Belgium (Piessens et al., 2011, Supré et al., 2011), and the Netherlands (Sampimon et al., 2009b), but would differ from those of studies carried out in Sweden (Thorberg et al., 2009, Waller et al., 2011) and Finland (Taponen et al., 2006). Remaining NCDF CNS isolates speciated by gene sequencing were found to be mainly *Staphylococcus simulans* (24.0%), *Staphylococcus xylosus* (8.8%), *Staphylococcus haemolyticus* (4.9%), and 16 other CNS species (12.9%) (J.R. Middleton, unpublished data).

One drawback of this study was the consideration of the CNS retrieved from NCDF farms as one homogeneous group. As can be seen from the small sample of CNS isolates that could be speciated, the isolates studied could be further differentiated into a few groups that could potentially show a certain level of heterogeneity in term of incidence and elimination rates, as well as risk factors for these. Because of the large number of isolates involved, identification of the CNS isolates at the species level was not available when the analyses were carried out. Plans for speciation of a larger sample of the NCDF CNS isolates have been laid and, in future research, this issue will be resolved. The present study should, therefore, be regarded as an exploratory study on the epidemiology of CNS as a group, while keeping in mind the possible heterogeneity of the isolates that constitute this

group. In addition, since CNS IMI duration or persistence could not be precisely established, this important aspect was not addressed in this study. The presented study was strictly focused on acquisition and elimination of CNS IMI over 3-week periods and on presence of CNS IMI.

Manageable Risk Factors

Many management practices were associated with the odds of having a prevalent CNS IMI. It is important to realize that these associations can only be mediated by an effect on CNS IMI incidence, elimination, or both. In addition, when measures of disease prevalence on their own are used, it is difficult to identify the correct time-order of occurrence between exposure and disease, and this can potentially lead to the identification of spurious associations. For these reasons, less consideration should be given to management practices associated solely with CNS IMI prevalence in particular or with only one outcome in general. For a thorough interpretation of the study's results, the authors suggest consideration of a holistic analysis and interpretation of associations with all 3 outcomes jointly. A conceptual chart of the associations between manageable risk factors and prevalence, incidence, and elimination of CNS IMI based on the results from Tables XIV, XV, and XVI is presented in Fig. 7, and should help the reader to bridge this gap.

As a starting point, it is worth mentioning that all risk factors associated with CNS IMI incidence were also associated with IMI prevalence. Conversely, a few of the risk factors associated with CNS IMI prevalence were not associated with IMI incidence. These differences may be explained, in part, by the higher power of the study for the prevalence dataset for which the number of observations and the distribution of the outcome were superior. Only one of the management practices studied - the type of bedding used in stalls or pens - showed similar associations with all 3 outcomes. Matos et al. (1991) have already reported disparities in bacterial load between bedding types and between fresh and used bedding. These researchers observed different distributions of staphylococci species among bedding types and reported these species as common in the cows' environment. Results from the present study suggest that bedding type plays a substantial role in CNS

epidemiology and, based on these previously published results, this role is probably mediated through differential selection of CNS species that are more or less competent at causing IMI. When compared to straw bedding, the use of sand bedding showed a desirable association with all 3 outcomes. In the literature, sand bedding has been consistently associated with lower SCC (Dufour et al., 2011b). Compared to organic bedding, very little substrate is available to support bacterial growth in an inorganic bedding, such as sand, and this may explain the lower IMI incidence and prevalence observed. Given that the odds of IMI elimination was greater for sand bedding, it also suggests that more poorly host-adapted CNS species or strains would be found in the environment of sand bedded barns. Our results also suggest that, among the organic bedding used, wood-based product would support either a lower quantity of CNS, less well host-adapted CNS species, or both. This is supported by results from Matos et al. (1991) who reported generally decreasing bacterial counts between hay, straw, and sawdust beddings as well as different CNS species populations between bedding types. In their study, the very different CNS populations found in alfalfa hay could explain the lower odds of IMI elimination observed in the present study.

In this study, quarters of cows that had access to pasture during the sampling period had lower IMI incidence and prevalence, which suggests a lower CNS infection pressure from pasture compared to confinement housing. These results are in contrast with those of Sampimon et al. (2009b) who found a higher herd CNS prevalence in herds where cows had access to pasture during the outdoor season. In that Dutch study, however, the yearly herd CNS prevalence was used as the outcome rather than the seasonal prevalence. The direct impact of pasture access, therefore, would be difficult to evaluate. In addition, it is likely that the very different weather and pasture conditions prevailing in the Netherlands compared to Canada could have led to these different observations.

The only other manageable risk factor associated with at least 2 of the studied outcomes was to provide a bonus for milk quality to the persons milking. It is difficult, however, to clearly evaluate the direct effect of such practice. Providing a bonus for milk

quality could, for instance, motivate the milkers to be more thorough and to follow more closely the recommended milking procedures, which would help prevent acquisition of new CNS IMI. The association seen would then be an indirect effect of this practice. On the other hand, providing such bonus could also be an indication of a more proactive attitude toward udder health in general, which would, in turn, lead to a greater adoption of other recommended practices. The association observed would then be a spurious association resulting from residual confounding by general attitude toward udder health.

A few manageable risk factors related to maternity pen management, cow cleanliness, purchase habits, and monitoring of udder health were associated solely with CNS IMI elimination. Further investigation into these possible risk factors should be undertaken before drawing any conclusions. Similarly, some practices related to milking procedures and maternity pen management were associated with IMI prevalence exclusively. Again, it is recommended that caution be used in drawing conclusions in these cases.

Two cornerstones of every mastitis control program, blanket dry cow therapy (**DCT**) and PMTD, were already used by a vast majority of the NCDF herds (88% for blanket DCT, and 99% for PMTD). Because of the low number of dairy producers not using these practices, the power to find significant associations between CNS IMI outcomes and blanket DCT or PMTD was limited. These practices should certainly not be rejected as potential important risk factors for CNS IMI based on the study's results. One should instead consider the manageable risk factors identified in this study as practices that could be use to control CNS IMI in herds already using blanket DCT and PMTD.

Finally, as mentioned before, it is still unclear whether or not CNS IMI are indeed detrimental to udder health. The SCC increases that have been generally reported for CNS IMI, though, seem to indicate that prevention of these IMI, at least in low BMSCC herds, is a cautious approach. In addition, most of the manageable risk factors for CNS IMI identified in this study, have shown desirable association in previous studies with other

measures of udder health. It would, therefore, be very unlikely that implementing these practices to control CNS IMI would result in a general deterioration of udder health.

Potential Bias

Like most exploratory studies, many potential biases may have led to the observed estimates of association. First of all, the herds selected were a convenience sample of Canadian dairy herds and, although they shared some similar attributes with the Canadian dairy herd population (Reyher et al., 2011), they may have differed from the target population in terms of CNS IMI burden or of management practices used. The resulting selection bias would affect estimates of CNS IMI prevalence, incidence, and elimination. It would, however, be much less likely to affect estimates of association between manageable risk factors and CNS IMI outcomes.

Secondly, although an effort was made to adjust for the most obvious confounders, it is likely that residual confounding still may bias the presented estimates to some extent. In a previously published study on manageable risk factors associated with *S. aureus* IMI (Dufour et al., 2011a), however, and using an extended and thorough investigation procedure to identify confounding, very few of the theoretically identified confounders were actually modifying the reported estimates by a significant amount (S. Dufour, unpublished data). So, in the opinion of the authors, although the direction of residual confounding bias is unpredictable, its magnitude is likely to be small.

Finally, despite the use of a latent class model approach to adjust the presented estimates for disease misclassification, and despite the use of Se and Sp thresholds for explanatory variables, a limited degree of misclassification bias probably remains. The level of control of misclassification bias that can be achieve using the latent class model approach, or any other misclassification adjustment approach, is directly related to the exactitude of the misclassification parameters (the Se and Sp) chosen (Lash et al., 2009). In this study, since the Se and Sp estimates were obtained from an internal validation study using a sample of the studied CNS isolates, the misclassification parameters used are likely

to be very close to the true Se and Sp values. Any remaining misclassification bias should, therefore, be fairly small.

Conclusions

Like a number of infectious diseases, prevention seems to be the key to long-term CNS IMI control. When an outcome is measured with an obviously imperfect diagnostic procedure, such as bacteriological culture for CNS, determining the direction and magnitude of the resulting bias on estimates of prevalence, incidence, elimination, or on associations with risk factors rapidly becomes intractable. In these situations, using a technique accounting for the test limitations would provide better estimates and would improved comparability between studies. In herds already employing blanket DCT and PMTD, many additional practices can be implemented to prevent acquisition of new CNS IMI. These practices seemed to be mainly related to management of the environment of the cow such as bedding condition or pasture access.

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Tables

Table XIII. Prior distributions used in the latent class model for bacteriological culture sensitivity (Se) and specificity (Sp).

Analysis	Param.[1]	Distribution	Mean[2]	Limits[3]	
				Lower	Upper
Incidence and elimination	Se	Beta(165, 39.0)	0.81	0.76	0.86
	Sp	Beta(145, 22.5)	0.87	0.82	0.92
Prevalence					
Series with 1 culture result	Se	Beta(165, 39.0)	0.81	0.76	0.86
	Sp	Beta(145, 22.5)	0.87	0.82	0.92
Series with 2 results[4]	Se	Beta(92, 4.0)	0.96	0.91	1.00
	Sp	Beta(174, 55.5)	0.76	0.71	0.81
Series with 3 results[4]	Se	Beta(68, 1.5)	0.98	0.93	1.00
	Sp	Beta(172, 89.0)	0.66	0.61	0.71

[1] Parameter estimated.

[2] All distributions were centered on the parameter estimate obtained using CBMRN isolates and reported in Dohoo et al. (2011)

[3] Lower and upper truncation of the distributions were implemented to avoid selection of less probable and sometimes inappropriate Se and Sp combinations and improve MCMC convergence. Lower and upper limits correspond to the parameter estimate reported in Dohoo et al. (2011) ± 5 percentage-points.

[4] Whenever CNS IMI status were determined using 2 or 3 bacteriological culture results interpreted in parallel, the Se and Sp estimates reported in Dohoo et al. (2011) were adjusted accordingly.

Table XIV. Final multivariable cross-classified hierarchical model of the relationship between manageable risk factors and odds of acquisition of new coagulase-negative staphylococci (CNS) IMI without and with adjustment for outcome misclassification.

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Housing type[b]						
Tie-stall	Ref	Ref	Ref	Ref	Ref	Ref
Free-stall	0.97	0.75	1.3	0.91	0.54	1.5
Bedded pack barn	0.72	0.46	1.1	0.51	0.18	1.3
Outside access						
No outside access	Ref	Ref	Ref	Ref	Ref	Ref
Access to exercise yard	0.92	0.63	1.4	0.81	0.40	1.6
Access to pasture	0.71*	0.61	0.81	0.52*	0.38	0.70
Type of bedding						
Straw	Ref	Ref	Ref	Ref	Ref	Ref
Sand	0.51*	0.33	0.78	0.27*	0.10	0.64
Wood products	0.73*	0.57	0.94	0.55*	0.31	0.90
Hay	1.0	0.58	1.8	1.0	0.36	3.0
Wood and straw	0.90	0.72	1.1	0.84	0.55	1.3
Milkers receive bonus for milk quality	0.59*	0.36	0.96	0.33*	0.11	0.91
% of clinical mastitis (CM) cases treated						
< 50%	Ref	Ref	Ref	Ref	Ref	Ref
50 to 90%	0.88	0.66	1.2	0.76	0.43	1.3
≥ 90%	1.3	0.98	1.6	1.6	0.98	2.7

[a] Median odds ratio estimate

[b] Variable kept in the model as confounding variable

* OR statistically significant (95% credibility interval not including the null value)

Table XV. Final multivariable cross-classified hierarchical model of the relationship between manageable risk factors and odds of elimination of coagulase-negative staphylococci (CNS) IMI without and with adjustment for outcome misclassification.

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Housing type[b]						
Tie-stall	Ref	Ref	Ref	Ref	Ref	Ref
Free-stall	1.2	0.94	1.6	1.9	0.80	4.4
Bedded pack barn	1.2	0.79	2.0	2.0	0.51	8.6
Type of bedding						
Straw	Ref	Ref	Ref	Ref	Ref	Ref
Sand	1.7*	1.1	2.5	4.9*	1.4	21.0
Wood products	1.1	0.92	1.4	1.6	0.79	3.2
Hay	0.91	0.62	1.3	0.67	0.18	2.3
Wood and straw	1.5*	1.2	1.8	3.3*	1.7	7.9
Lower leg cleanliness score						
Very clean	Ref	Ref	Ref	Ref	Ref	Ref
Clean	0.90	0.73	1.1	0.82	0.42	1.6
Dirty	1.1	0.90	1.4	1.7	0.83	3.6
Very dirty	1.4	1.0	1.8	2.9*	1.2	8.1
Distance neckrail-curb						
< 1.7m	Ref	Ref	Ref	Ref	Ref	Ref
1.7 to 1.8m	1.2	0.94	1.5	1.8	0.83	4.3
1.8 to 1.9m	0.93	0.65	1.3	0.76	0.23	2.4
>1.9m	0.91	0.69	1.2	0.74	0.30	1.8
Type of bedding in maternity pens (MP)						
Wood products	Ref	Ref	Ref	Ref	Ref	Ref
Straw	0.59*	0.45	0.76	0.20*	0.07	0.51
Hay	0.45	0.11	1.8	0.06	<0.01	6.6
Wood products and straw	0.65*	0.47	0.90	0.26*	0.08	0.82
Bedding added to MP						
≥ once/d	Ref	Ref	Ref	Ref	Ref	Ref
once/d to once/mo	0.72*	0.60	0.80	0.37*	0.18	0.69
< once/mo	0.72	0.45	1.2	0.46	0.09	2.0
After every calving	0.70*	0.54	0.92	0.34*	0.13	0.81
As needed	1.7	0.58	5.0	9.4	0.24	>100.0

Table XV. (Continued)

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Measures milk conductivity	0.57*	0.42	0.76	0.16*	0.05	0.41
Ration balanced based on forage analyses	0.63	0.47	1.1	0.32	0.04	1.6
Purchase habits in preceding 6 mo						
Never buys cattle	Ref	Ref	Ref	Ref	Ref	Ref
Usually buy cattle but not in last 6 mo	0.42	0.17	1.1	0.04	<0.01	1.0
Purchased only heifers	1.2	0.94	1.5	1.8	0.84	4.0
Purchased cows	1.3*	1.1	1.5	2.3*	1.4	3.9

[a] Median odds ratio estimate

[b] Variable kept in the model as confounding variable

[c] Median odds ratio estimate and 2.5th and 97.5th percentiles are presented per increase of 30 days in milk

* OR statistically significant (95% credibility interval not including the null value)

Table XVI. Final multivariable cross-classified hierarchical model of the relationship between manageable risk factors and odds of a prevalent coagulase-negative staphylococci (CNS) IMI without and with adjustment for outcome misclassification.

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Housing type[b]						
Tie-stall	Ref	Ref	Ref	Ref	Ref	Ref
Free-stall	1.2	0.83	1.7	1.5	0.78	2.9
Bedded pack barn	0.76	0.41	1.4	0.73	0.23	2.5
Herd mean SCS in preceding 24 mo[b]	1.1	0.83	1.4	1.3	0.80	2.1
Outside access						
No outside access	Ref	Ref	Ref	Ref	Ref	Ref
Access to exercise yard	1.3	0.85	1.9	1.5	0.75	3.2
Access to pasture	0.80*	0.68	0.93	0.71*	0.52	0.97
Type of bedding						
Straw	Ref	Ref	Ref	Ref	Ref	Ref
Sand	0.58*	0.36	0.96	0.39*	0.16	0.96
Wood products	0.70*	0.54	0.92	0.48*	0.29	0.79
Hay	3.9*	1.7	8.7	7.8*	2.0	37.3
Wood and straw	0.72*	0.56	0.91	0.56*	0.36	0.87
Type of bedding by herd SCS						
Straw by herd SCS	Ref	Ref	Ref	Ref	Ref	Ref
Sand by herd SCS	0.93	0.49	1.8	0.95	0.25	3.6
Wood products by herd SCS	1.0	0.76	1.4	1.1	0.65	1.9
Hay by herd SCS	0.21*	0.11	0.41	0.11*	0.03	0.29
Wood and straw by herd SCS	0.97	0.69	1.4	0.91	0.48	1.8
Distance neckrail-curb						
< 1.7m	Ref	Ref	Ref	Ref	Ref	Ref
1.7 to 1.8m	0.85	0.58	1.2	0.63	0.30	1.3
1.8 to 1.9m	0.69	0.40	1.2	0.43	0.15	1.1
>1.9m	1.1	0.73	1.7	0.99	0.43	2.1
Cows left >7d in MP after calving	0.38*	0.18	0.82	0.12*	0.02	0.57
Milkers receive bonus for milk quality	0.59	0.33	1.0	0.27*	0.08	0.89
S. aureus cows milked last or with a specific unit	1.3	0.95	1.8	1.6	0.89	3.3

Table XVI. (Continued)

Independent variable	Non-adjusted estimates			Misclassification adjusted estimates		
	OR[a]	OR percentiles		OR[a]	OR percentiles	
		2.5th	97.5th		2.5th	97.5th
Teat drying method						
No drying	Ref	Ref	Ref	Ref	Ref	Ref
Paper towels	0.67*	0.51	0.89	0.51*	0.32	0.85
Reusable cloth towels	0.63*	0.46	0.86	0.39*	0.21	0.73
Feed total mixed ration	1.3*	1.1	1.7	1.6	1.0	2.6
% of clinical mastitis (CM) cases treated						
< 50%	Ref	Ref	Ref	Ref	Ref	Ref
50 to 90%	0.85	0.63	1.1	0.70	0.42	1.2
≥ 90%	1.2	0.88	1.5	1.4	0.85	2.2
Herd SCS at beginning of sampling period	1.2	0.99	1.4	1.3	0.96	1.7

[a] Median odds ratio estimate

[b] Variable kept in the model as confounding variable

* OR statistically significant (95% credibility interval not including the null value).

Figures

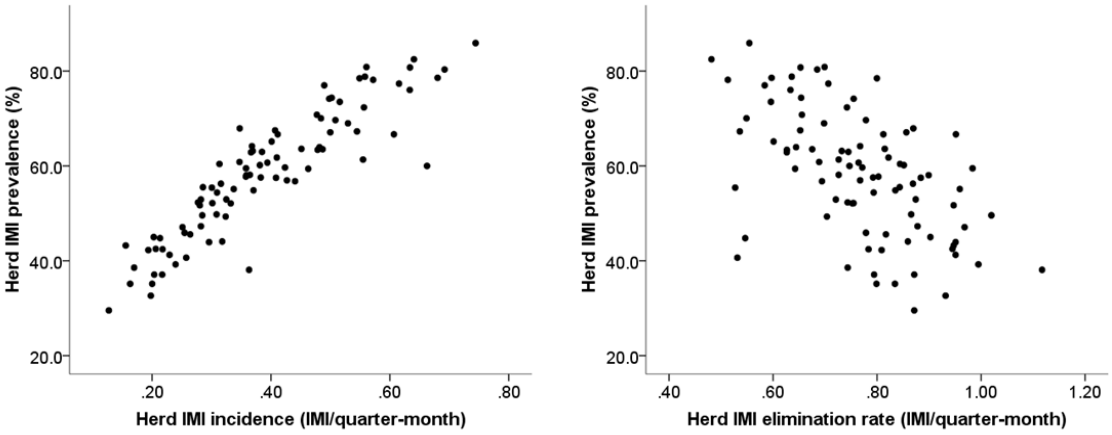

Figure 6. Scatter plots of the herd coagulase-negative staphylococci (CNS) IMI prevalence against herd IMI incidence and elimination rates.

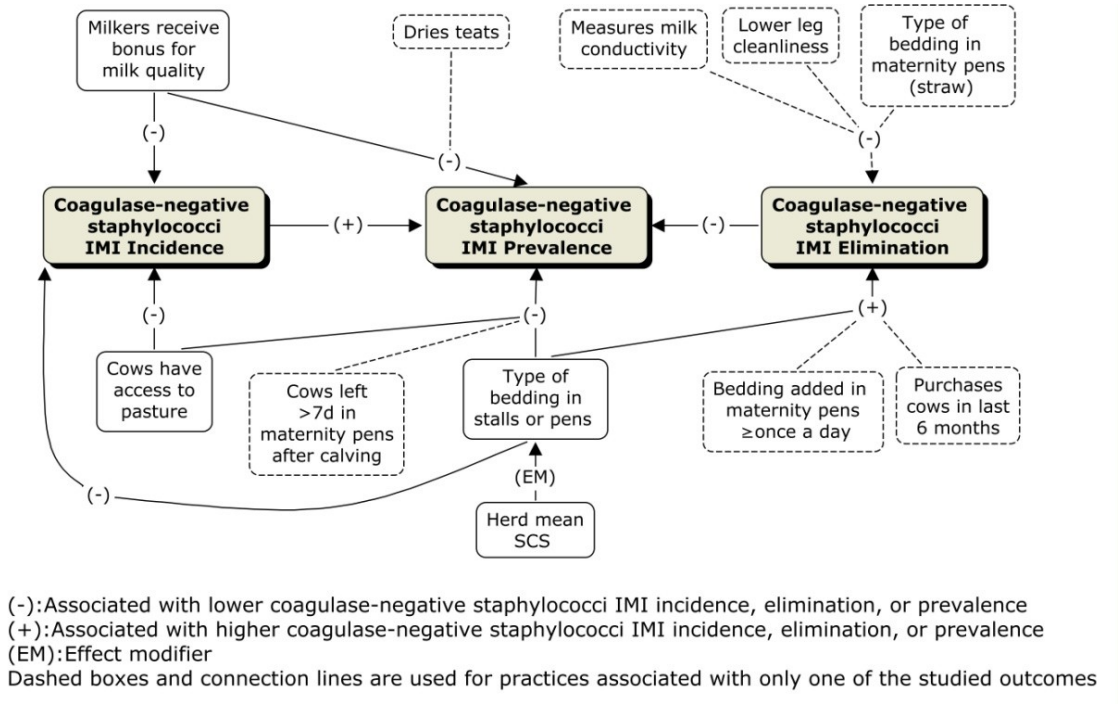

Figure 7. Conceptual chart of associations between manageable risk factors and coagulase-negative staphylococci (CNS) IMI incidence, elimination, and prevalence.

Discussion générale

L'objectif de cette thèse était d'approfondir les connaissances sur l'épidémiologie des IIM d'importance durant la période lactée chez les vaches laitières canadiennes. Bien que l'orientation principale de cette thèse était avant tout centrée sur les IIM, plusieurs aspects méthodologiques ont également dû être traités et ont parfois demandé le développement de nouvelles méthodes ou l'adaptation de méthodes existantes. L'utilisation de ces méthodes a permis d'apporter plus de robustesse aux analyses épidémiologiques sur les IIM et certaines de ces méthodes pourraient certainement être intégrées de manière courante dans l'étude de ces infections. Le but de cette discussion est de souligner et mettre en relation les principaux résultats ainsi que de discuter des forces et faiblesses de cette étude.

Pratiques de gestion et CCS

Dans la première partie de cette thèse une revue systématique de littérature sur l'association entre les pratiques de gestion utilisées à la ferme et le CCS a été réalisée. L'objectif premier était d'identifier les pratiques de gestion ayant démontré une association constante avec une mesure générale de la santé de la glande mammaire. Cette première étude était également nécessaire afin d'identifier les pratiques de gestion qui pourraient par la suite être examinées dans les chapitres subséquents de cette thèse.

Avant même de répondre à l'objectif initialement fixé, cette étude à d'abord permis de souligner certains traits récurrents des recherches effectuées dans les 30 dernières années en santé de la glande mammaire. D'abord, malgré le fait que les mesures de CCS soient habituellement collectées de manière longitudinale, souvent mensuellement, pratiquement tous les manuscrits sélectionnés avaient adopté un dessin d'étude transversale. De plus, une très grande hétérogénéité quant à la définition de la variable dépendante est notée. Cette hétérogénéité peut en partie être expliquée par des différences régionales ou historiques en santé de la glande mammaire. Par exemple, la valeur seuil pour distinguer

les troupeaux à bas CCS des troupeaux à CCS élevé varie certainement en fonction des valeurs de CCS généralement rencontrées dans un pays ou une région ainsi qu'en fonction de l'année de réalisation de l'étude. Cette hétérogénéité dans la définition de la variable dépendante est cependant aussi fréquemment rencontrée entre différentes études réalisées sur un même agent pathogène, dans une même région géographique et dans une période de temps relativement restreinte. Elle est attribuable, dans ce cas, à l'absence de consensus dans la communauté scientifique quant à la définition de ce qui constitue une IIM. En l'absence d'une procédure diagnostique pouvant faire office "d'étalon-standard'', ce manque de consensus est certainement une faiblesse importante de la recherche en santé de la glande mammaire. Finalement, pour la majorité des études sélectionnées, un questionnaire était utilisé afin de mesurer les pratiques de gestion utilisées sur les fermes participantes. Hors, pratiquement aucune de ces études ne semble avoir réalisé une validation de l'outil de mesure utilisé et, dans toutes les analyses présentées, une mesure parfaite des pratiques de gestion en place sur les fermes étudiées est supposée de manière implicite.

L'analyse des résultats de cette revue systématique a permis d'identifier un nombre relativement limité de pratiques pour lesquelles une association récurrente et constante avec la santé de la glande mammaire pouvait être démontrée. D'autres pratiques faisant l'objet de rapports anecdotiques ou mitigés ont également pu être identifiées. De plus, plusieurs pratiques de gestion habituellement recommandées dans les programmes de contrôle de la mammite semblent être appuyées par un niveau relativement limité de preuve de leur efficacité. Dans plusieurs cas, les associations observées pourraient être le résultat d'un facteur confondant non pris en compte par le dessin d'étude ou les analyses épidémiologiques. Les attitudes, comportements et connaissances des producteurs laitiers, entres autres choses, ont été associés dans la littérature aux pratiques de gestion utilisées ainsi qu'à la santé de la glande mammaire et pourraient possiblement confondre certaines des associations rapportées (Barkema et al., 1999; Jansen et al., 2009).

Finalement, cette revue de littérature a été réalisée à l'intérieur d'un cadre bien précis à l'aide de critères de recherche et d'inclusion très spécifiques. Il ne s'agit en aucun cas d'une revue exhaustive des pratiques de gestion associées à la santé de la glande mammaire. Par exemple, plusieurs pratiques de gestion ont, par le passé, été associées à la prévalence ou à l'incidence d'agents pathogènes spécifiques de la mammite sans être directement associés au CCS. Les études d'efficacité de produits utilisés pour la désinfection post-traite des trayons en est un exemple flagrant. De plus, beaucoup de pratiques ont été associées à l'incidence de mammite clinique, une composante importante de la santé de la glande mammaire, mais ayant un impact limité sur le CCS du troupeau.

Développement et validation d'un questionnaire bilingue

Malgré le nombre élevé d'études sur la santé de la glande mammaire ayant eu recours à un questionnaire afin de mesurer les pratiques de gestion utilisées à la ferme, aucun questionnaire validé n'a été publié sur le sujet. Comme toute procédure diagnostique, les questionnaires épidémiologiques possèdent une précision (la répétabilité) et une validité intrinsèques. L'objectif de cette partie de la thèse était donc de développer et valider, à partir des résultats de la revue systématique de littérature, un outil permettant de mesurer les pratiques utilisées sur les fermes laitières canadiennes. De plus, l'équivalence entre les versions anglaise et française du questionnaire devait être démontrée.

La littérature scientifique traite abondamment des méthodes permettant d'évaluer la répétabilité et la validité de questionnaires épidémiologiques. Par contre, quoique les méthodes de traduction de ces instruments aient été abordées dans la littérature, il existe relativement peu de lignes directrices quant à l'évaluation de l'équivalence de versions multilingues de questionnaires épidémiologiques. Dans le cas d'un questionnaire épidémiologique, l'équivalence inter-langage de chacun des éléments constituant le questionnaire devra être démontrée. En ce sens, l'approche utilisée permettant de comparer les mesures de répétabilité entre les langues et dans la même langue et obtenues d'un

échantillon d'individus bilingues répondant de multiple fois aux différentes versions du questionnaire semble appropriée. Une approche similaire mais n'évaluant que la répétabilité entre les langues à déjà été suggérée (Carlson, 2000). Une telle approche ne permettait pas, cependant, de différencier un item ayant une répétabilité basse, d'un item mal traduit. À notre connaissance, il s'agit de la première mention dans la littérature de l'utilisation d'une approche telle que celle proposée dans ce chapitre.

Cette étude a permis le développement d'un outil bilingue de mesure des pratiques de gestion généralement satisfaisant. Elle a cependant surtout permis d'identifier les éléments de cet outil qui étaient inadéquats pour les analyses épidémiologiques planifiées. Dans plusieurs cas, une recatégorisation de certains éléments permettait de remédier à certaines déficiences au détriment, cependant, des subtilités initialement recherchées. Dans d'autre cas, des éléments ont simplement dû être écartés des analyses subséquentes. La validation de ce genre d'instrument de recherche est une tâche fastidieuse mais apporte une compréhension inestimable des données recueillies. Ce travail a permis d'identifier plusieurs points utiles au développement de tels outils :

- Un nombre élevé de pré-tests devraient être réalisés par un interviewer attentif avant l'administration de l'outil à la population cible. Selon notre expérience, 20 administrations auraient permis de corriger plusieurs items problématiques;
- L'utilisation d'une méthode reconnue de traduction ne constitue pas une garantie d'équivalence des différentes versions d'un questionnaire. Il ne s'agit que d'une évaluation de la constance du processus de traduction et l'équivalence des versions devrait être évaluée formellement;
- Une proportion relativement élevée (près de 30%) des producteurs laitiers modifie une ou plusieurs des pratiques de gestion en place sur leur ferme sur une période de seulement six mois. Dans la situation où les données recuellies serviront à établir les expositions en place à la ferme sur une période prolongée, certaines pratiques devraient donc être mesurées à plusieurs reprises afin de détecter ces modifications;
- La répétabilité des questions portant sur les attitudes et les motivations des producteurs laitiers est, en général, faible. Ces attributs ont tendance à varier d'une

journée à l'autre pour un même individu et la manière dont il rapporte lui-même ces attributs varie également. L'utilisation de ces mesures plutôt approximatives dans le but de contrôler les biais de confusion, ne permettra pas de complètement contrôler ces biais.

Les résultats de cette étude ont également permis d'obtenir un estimé des pratiques de gestion en place sur les fermes laitières canadiennes. Un point clé à retenir est que plusieurs des pratiques de gestion recommandées depuis parfois plus de 30 ans et pour lesquelles les liens avec le CCS et la santé de la glande mammaire sont fortement corroborés par la littérature scientifique, ne sont toujours pas adoptées par la majorité des producteurs laitiers canadiens. Pourtant, plusieurs de ces pratiques sont peu dispendieuses et peuvent facilement être implantées à la ferme. Par exemple, seulement 56% des producteurs laitiers de la NCDF portent des gants lors de la traite. Seulement 53% tirent les premiers jets avant la traite et moins de 66% utilisent la désinfection pré-traite des trayons. Lors de l'administration du questionnaire, les producteurs interviewés semblaient être au courant de l'existence de ces pratiques. Cependant, les seules pratiques qui semblent avoir été adoptées par une large proportion de producteurs sont la désinfection des trayons après la traite (99%) et l'administration universelle d'antibiotiques au moment du tarissement (88%). Ces estimés obtenus des producteurs de la NCDF en 2007 et 2008 sont tous relativement comparables à ceux obtenus à partir d'un échantillon aléatoire de fermes canadiennes de 2004 à 2006 (Olde Riekerink et al., 2010). Étant donné le faible niveau d'adoption de plusieurs pratiques importantes, il semble évident que de nouvelles approches devront être employées afin de convaincre une plus large proportion de producteurs laitiers d'adopter les pratiques recommandées.

Facteurs de risques modifiables associés aux IIM

Dans cette partie de la thèse, l'épidémiologie des IIM causées par les *S. aureus* et les SCN, des IIM d'importance pour l'industrie laitière canadienne, est explorée de même

que les pratiques de gestion modifiables associées à ces IIM. L'objectif de ces études était d'identifier les pratiques associées à l'incidence et à l'élimination de ces IIM qui sont elles-mêmes les déterminants de la prévalence d'IIM. Cependant, afin de pouvoir clairement positionner et différencier les résultats de ces études longitudinales de ceux des études de prévalence fréquemment réalisées en santé de la glande mammaire, l'analyse des pratiques associées à la prévalence d'IIM a également été réalisée. Un volet important de ces analyses était d'ajuster les estimés d'association pour les biais de confusion les plus importants de même que d'identifier les conditions présentes à la ferme et ayant le potentiel de moduler l'effet de ces pratiques. Un dernier volet était de contrôler lorsque nécessaire les biais dus à l'erreur de classification des pratiques en place à la ferme ou encore du statut infectieux des quartiers étudiés.

Les troupeaux recrutés pour cette étude avaient déjà adopté, dans une large proportion, la désinfection des trayons post-traite (99%) et le traitement universel des quartiers au tarissement (88%), deux composantes essentielles de tout programme de contrôle de la mammite. Les pratiques de gestion identifiées dans cette étude devraient donc être considérées comme des pratiques supplémentaires qui pourraient être utilisées à la ferme afin de contrôler les IIM à *S. aureus* et à SCN, lorsque ces mesures sont déjà en place.

Pour ce qui est des IIM à *S. aureus*, la plupart des pratiques de gestion significativement associées à ces IIM sont liées aux techniques de traite, à la prévalence de quartiers infectés dans le troupeau, ainsi qu'à la gestion des mammites cliniques. Entre autres, le port de gants durant la traite, la désinfection pré-traite des trayons et une bonne condition du bout du trayon démontrent tous des associations intéressantes et désirables avec ces IIM. Ces résultats concordent avec les connaissances actuelles sur l'épidémiologie des IIM à *S. aureus*. De plus, il est intéressant de noter que les estimés d'association des pratiques associées à l'incidence d'IIM sont d'un ordre de grandeur considérable. En assumant que les relations observées sont effectivement causales, ces résultats suggèrent que l'adoption de certaines de ces pratiques pourraient encore réduire de

plus de moitié, dans bien des cas, le nombre de nouvelles IIM à *S. aureus* sur les fermes laitières pratiquant déjà la désinfection post-traite des trayons et le traitement universel des quartiers au tarissement. Un tel potentiel d'amélioration justifie certainement les efforts requis pour amener les producteurs laitiers à changer leur mentalité et à adopter ces pratiques additionnelles qui sont souvent peu coûteuses.

Peu d'estimés de l'incidence d'IIM à SCN ont été rapportés dans la littérature. L'incidence moyenne observée de 0.29 nouvelles IIM à SCN/quartier-mois est extrêmement élevée en comparaison aux incidences rapportées pour les autres agents pathogènes de la mammite. Cette incidence élevée est certainement une caractéristique importante de l'épidémiologie de ces IIM et semble être le déterminant le plus important de la prévalence d'infections à SCN dans les troupeaux étudiés. Peu de pratiques de gestion semblent être associées à la fois à plusieurs des variables dépendantes étudiées. La gestion de l'environnement des vaches laitières semble être un élément important pour le contrôle des IIM à SCN. Les litières de sable et de produits du bois paraissent mieux à même de contrôler les IIM à SCN en comparaison à la litière de paille (le type de litière le plus fréquemment utilisé dans les troupeaux de la NCDF). De même, l'accès au pâturage semble être associé à un risque plus faible d'acquisition et de prévalence d'IIM.

Comme pour beaucoup d'études portant sur les SCN, l'identification plus détaillée des espèces de SCN causant les IIM observées n'a pu être réalisée. Les différents SCN retrouvés ne partagent possiblement pas tous les mêmes déterminants épidémiologiques et cette simplification pourrait avoir donné naissance à, ou avoir faussé certaines des observations rapportées. L'étude présentée est une étude exploratrice sur les SCN en général. Avec les plans de spéciation des SCN de la NCDF déjà établis, de futures recherches portant sur l'épidémiologie de certaines espèces de SCN en particulier pourront venir préciser ces résultats.

L'approche holistique proposée pour l'interprétation des facteurs de risque associés à l'incidence, l'élimination, et à la prévalence d'IIM est certainement un point fort de cette partie de cette thèse. La littérature scientifique sur les analyses de facteurs de risque de la

mammite ou de d'autres domaines d'étude, repose habituellement sur l'identification des facteurs associés à un seul des déterminants de la maladie et, plus souvent qu'autrement, sur les associations avec la prévalence de maladie. Hors, ces études sur la prévalence peuvent mener à l'identification d'associations fallacieuses puisque l'ordre chronologique entre exposition et maladie n'est pas connu. De plus, un facteur de risque ne peut avoir un réel impact sur la prévalence d'IIM qu'en modulant leur acquisition, leur élimination, ou à la fois ces deux déterminants de la prévalence d'IIM. Il semble donc beaucoup plus approprié de considérer ensemble les associations avec ces trois mesures de la maladie plutôt qu'avec une seule, surtout ci celle-ci est la prévalence de maladie. Les pratiques associées uniquement à la prévalence d'IIM pourraient, néanmoins, êtres réellement associées, mais faiblement, à la fois à l'incidence et à l'élimination des IIM et donc avoir un impact réel sur la prévalence d'IIM. Inversement, il est intéressant de noter que tous les facteurs de risque associés à l'incidence d'IIM à *S. aureus* ou à SCN, le déterminant le plus important de la prévalence de ces IIM, étaient également associés à la prévalence de ces IIM. Ces résultats soulignent d'autant plus les pratiques de gestion associés à l'incidence d'IIM comme des éléments de contrôle important pour une réduction à long terme de la prévalence d'IIM. Ces résultats soulèvent également de nombreuses questions sur la validité des facteurs de risque habituellement identifiés à l'aide d'études de prévalence et viennent supporter le choix de l'approche d'interprétation holistique des résultats proposée pour ces études.

Une différence importante de cette étude, en comparaison à la littérature scientifique préalablement publiée sur le sujet, réside dans le traitement des biais de mauvaise classification. D'abord, la mauvaise classification du statut infectieux des quartiers demeure un point délicat en recherche sur la santé de la glande mammaire. L'absence "d'étalon-standard" pour le diagnostique des IIM a été un obstacle majeur dans ce domaine de recherche et explique, en partie, l'absence de consensus de la communauté scientifique quant à la définition de ce qui constitue une IIM. Des études récentes ont permis de contourner en partie ce problème et des estimés de la Se et de la Sp de la culture bactériologique du lait obtenus à partir d'isolats de la NCDF et ce pour différentes

définitions d'IIM étaient disponibles (Andersen et al., 2010; Dohoo et al., 2011). Pour les IIM causées par certains pathogènes tels *S. aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, ou *Escherichia coli*, qui ont une faible prévalence et pour lesquels la spécificité de la culture bactériologique du lait est excellente (Dohoo et al., 2011), l'ampleur de ce biais de mauvaise classification est extrêmement limitée. Pour d'autres agents pathogènes plus fréquents, tels les SCN, les *Corynebacterium* spp et les enterocoques, la mauvaise classification du statu infectieux du quartier résultant des limites inhérentes à la culture bactériologique du lait a certainement un impact considérable sur la précision des mesures d'incidence, d'élimination, de prévalence et sur les mesures d'association avec les expositions. Dans cette étude, les coefficients de régression étaient en général sous-estimés lorsque les biais de mauvaise classification des IIM n'étaient pas pris en considération. Un point important à noter est que les estimés de déviation standard étaient également sous-estimés. Dans plusieurs cas, le fait d'ignorer la mauvaise classification des IIM menait à des erreurs de Type I ou II. En ce sens, l'utilisation d'une méthode permettant de prendre en compte les limitations de la procédure diagnostique est certainement souhaitable pour ces agents pathogènes. Plusieurs méthodes relativement simples et efficaces ont été développées afin de corriger les estimés de mesure de fréquence des maladies (Lash et al., 2009). La méthode utilisée dans le chapitre sur les SCN et proposée par McInturf et al. (2004) est intéressante en ce qu'elle permet également de contrôler le biais dans les estimés d'association avec les expositions. De plus, les structures de dépendance fréquemment rencontrées en recherche sur la santé de la glande mammaire peuvent être traitées aisément dans ces modèles. Finalement, l'imprécision autour des estimés de Se et Sp peut aussi être facilement pris en compte. Ce dernier élément pourrait s'avérer important lorsque les mesures de Se et de Sp de la culture bactériologique du lait ont été obtenues d'une population d'isolats différente de celle étudiée. Malgré tous ces avantages, il s'agit, à notre connaissance, de la première utilisation de cette méthode en santé de la glande mammaire. Plus souvent qu'autrement, les biais de mauvaise classification ne sont que discutés qualitativement dans ce domaine de recherche.

De même, le biais dans les estimés d'association résultant de la mauvaise classification des expositions a déjà été largement discuté dans la littérature (Gustafson 2004; Höfler, 2005; Gustafson et Greenland, 2006). Contrairement à la croyance, la direction de ce biais est souvent difficilement prévisible. De plus, ce biais peut mener à des intervalles de confiance parfois faussement étroits, parfois faussement larges, augmentant ainsi à la fois le risque d'erreur de Types I et II. Bien que beaucoup d'études d'analyse de facteurs de risque mesurent les expositions à l'aide de questionnaires, les limites de l'outil de mesure sont, la plupart du temps, inconnues ou ignorées. Le travail de validation du questionnaire réalisé dans cette étude est donc un volet extrêmement important de celle-ci en ce qu'il a permis de sélectionner ou transformer les variables indépendantes afin de minimiser ces biais.

Finalement, un élément du biais de mauvaise classification des IIM n'a pu être traité dans cette étude à l'aide des méthodes proposées. Il s'agit du biais de sélection résultant de la classification imparfaite du statut infectieux des quartiers au moment du prélèvement du premier échantillon de lait. En effet, les limites de la culture bactériologique du lait ont également un impact sur l'identification des quartiers à risque de devenir infectés ou à risque d'éliminer une IIM. Lors des analyses sur les facteurs de risque associés à l'incidence ou à l'élimination des IIM, une certaine proportion des quartiers sélectionnés ont certainement été incorrectement recrutés ou exclus. L'impact de ce biais de sélection sur les mesures d'association est, cependant, certainement limité en comparaison au biais de mauvaise classification préalablement discuté. Une étude est présentement en cours afin d'évaluer l'impact relatif de ce biais et d'identifier les situations où une mesure plus précise du statut infectieux initial des quartiers serait justifiée.

Conclusions

Cette étude a permis d'identifier les pratiques de gestion associées à certaines IIM d'importance dans les troupeaux canadiens, tout en suggérant de nouvelles approches pouvant être utilisées en recherche sur la santé de la glande mammaire. Les conclusions les plus importantes sont :

- La validation des questionnaires épidémiologiques utilisés afin de mesurer les expositions à des facteurs de risque hypothétiques est un aspect incontournable de l'utilisation de ces outils. La meilleure compréhension des données qui résulte de cette validation peut et doit être incorporée aux analyses épidémiologiques subséquentes;
- Outre la désinfection post-traite des trayons et le traitement universel des quartiers au tarissement, plusieurs pratiques supplémentaires pourraient être implantées sur les fermes laitières canadiennes. Ces pratiques sont souvent faciles à implanter et peu coûteuses, mais ont peu été adoptées jusqu'à présent par les producteurs laitiers canadiens;
- Pour certains agents pathogènes tels les SCN, le fait d'ignorer les limites de la culture bactériologique du lait entraine un biais considérable dans les estimés d'incidence, d'élimination et de prévalence, de même que dans les mesures d'association avec les expositions hypothétiques. Une approche à l'aide d'un modèle de variable à classe latente a permis de contrôler avec succès les biais de mauvaise classification résultants des limitations de la culture bactériologique du lait;
- Dans le cas des IIM à *S. aureus*, le port de gants durant la traite, la désinfection pré-traite des trayons, la bonne intégrité du bout des trayons et l'utilisation d'un protocole de réforme adéquat pour les vaches souffrant de mammite clinique pourraient permettre de réduire substantiellement l'incidence d'IIM par cet agent pathogène. L'effet de ces pratiques est

potentiellement médié par la prévention de la transmission des IIM d'un quartier à l'autre sur une même vache ou d'une vache à l'autre dans un troupeau. L'utilisation de stalles plus longues pourraient aussi potentiellement réduire le nombre de nouvelles IIM;

- Pour les IIM à SCN, l'utilisation de litière de sable ou de produits du bois et l'accès au pâturage, lorsque possible, sont des pratiques de gestion qui pourraient permettre de contrôler l'incidence de ces IIM. Il est important de noter que les pratiques de gestions associées à l'incidence de SCN étaient principalement liées à la gestion de l'environnement des vaches, ce qui vient supporter l'aspect environnemental de l'éthiologie d'une proportion importante de ces IIM.

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Annexe I. Udder health questionnaire (English)

A questionnaire designed to measure management practices used on Canadian dairy farms was developed by the Canadian Bovine Mastitis Research Network. This document is intended as a guide for future users. A few questions developed using the same described protocol but for which validity has not been tested are also included in the present document, validation of these questions is recommended.

General information:

The questionnaire was designed for an in-person interview format. The original instrument was split in two parts to limit the length of the interview. This questionnaire has been translated in French using a standardized translation method and the equivalence of the French and English versions has been tested.

Administration protocol:

Interviewer:

- Completely read each question as well as the answer choices to the interviewee.
- Care must be taken not to influence the producer's answers:
    - Keep the same voice tone while reading the questions and between the different choice of answers.
    - If a question is not understood by the interviewee, you can give a short clarification, such as definition of a term used, be careful not to favour an answer. Do not give examples while explaining.
    - Do not help the producer to choose the answer.
- Please answer each question in this questionnaire. If a question does not apply to the farm, for example a question concerning maternity pen and there is no maternity pen then check the ″Not applicable″ box.

Producer:

- Try to be precise in your answers. If you are not certain of the answer please consult your records before answering. Do not hesitate to add a comment if you feel that it is needed.
- Each part of this questionnaire should take you about 25 minutes to complete.
- All information will be treated as strictly confidential; your name and farm's identification number will not appear in any report.

First part:

1) Housing:

Milking cows

1) Type of **housing** for your **milking** cows:

- ☐ Tie-stall
- ☐ Free-stall
- ☐ Bedding-pack (i.e.: Straw-pack, sawdust-pack …)
- ☐ Other *(Please specify)*: _____
  _____

a) If you have a **free-stall** or **bedding pack barn**, how are the **passageways** cleaned?

- ☐ Not applicable
- ☐ Slatted floor
- ☐ Scraped
- ☐ Flushed with water
- ☐ Other *(Please specify)*: _____

b) If you have a **free-stall** or **bedding pack barn**, how many times a day are the passageways cleaned?

- ☐ Not applicable
- ☐ _____ times/day
  (Number)

2) Did your **milking cows** have access to pasture in the **last 12 months**?

- ☐ No, they are kept inside year-round
- ☐ No, but they had access to a grassed exercise yard (less than 5 acres per 100 cows)
- ☐ No, but they had access to a non-grassed (paved or dirt) exercise yard (less than 5 acres per 100 cows)
- ☐ Yes, they were on pasture from the month of _____ to the month of _____
  (Month) *(Month)*

3) What type of material is the **base** of your **milking** cows' stalls (or pens for bedding pack barn) made off? *(Check all that apply)*

- ☐ Concrete
- ☐ Sand
- ☐ Mattress (e.g.: Pasture mat…)
- ☐ Rubber mat
- ☐ Other *(Please specify)*: _____

4) What type of **bedding** is used for your **milking** cows' stalls (or pens for bedding pack barn)? *(Check all that apply)*

- ☐ Straw
- ☐ Sawdust
- ☐ Shavings
- ☐ Sand
- ☐ No bedding
- ☐ Other *(Please specify)*: _____

5) What type of **bedding management** do you use?

- ☐ I do not use any bedding
- ☐ I use a very low amount of bedding (**less than 2 cm deep**).
- ☐ I use deep bedding (**more than 2 cm deep**).
- ☐ Other *(Please specify)*: _____

6) How often do you **clean out manure** from your **milking** cows' stalls (or pens for bedding pack barn)?

- ☐ Never
- ☐ _____times/day *(Number)*
- ☐ Other *(Please specify)*: _____

7) How often do you **scrape out the dirty bedding** from your **milking** cows' stalls?

- ☐ Not applicable
- ☐ Never
- ☐ _____times/day *(Number)*
- ☐ _____times/week *(Number)*

a) If you do **scrape out dirty bedding** from your **milking** cows' stalls, **what part of the stall** do you scrape off?

- ☐ Not applicable
- ☐ Back third of the stall
- ☐ Back half of the stall
- ☐ The whole stall
- ☐ Other *(Please specify)*:_____

8) How often do you **add new bedding** to your **milking** cows' stalls (or pens for bedding pack barn)?

- ☐ Not applicable
- ☐ _____times/day
  (Number)
- ☐ _____times/week
  (Number)
- ☐ _____times/year
  (Number)

9) How often do you completely **remove and replace the bedding** in your **milking** cows' stalls (or pens for bedding pack barn)?

- ☐ Not applicable
- ☐ Never
- ☐ _____times/week
  (Number)
- ☐ _____times/month
  (Number)
- ☐ Other *(Please specify)*: _____

10) When you empty the stalls or pens completely, do you **wash or disinfect** them?
(Check all that apply)

- ☐ Not applicable
- ☐ Yes, pressure washed or brushed
- ☐ Yes, disinfected with _____
  (Name of the product)
- ☐ Yes, other *(Please specify)*: _____
- ☐ No

11) Do you add a product to limit growth of bacteria in your **milking** cows' bedding and/or stalls? (e.g.: Limestone …)

- ☐ Yes *(Please specify the product you are using and frequency of use)*: _____
  _____
- ☐ No

12) Do you use fly control methods during summer time? (e.g.: insecticide, traps ...)

- ☐ Yes
- ☐ No

Maternity pens

13) Do you have **maternity pens**?

- ☐ Yes, I have _____ maternity pens. *(Number)*
- ☐ No

a) Do you use maternity pens for **other purposes than calving** (i.e.: sick cows, lame cows …)?

- ☐ Not applicable
- ☐ Yes
- ☐ No

b) What **proportion of your calving** occurs in a maternity pen?

- ☐ Not applicable
- ☐ _____ % of the calving *(Number)*

c) How many **days** do your cows stay in the maternity pen?

- ☐ Not applicable
- ☐ _____ Days before calving and _____ days after calving *(Number)* *(Number)*

d) Do you keep **more then one cow** per maternity pen?

- ☐ Not applicable
- ☐ No
- ☐ Yes

e) What type of material is the **base** of your **maternity pens** made off? *(Check all that apply)*

- ☐ Not applicable
- ☐ Concrete
- ☐ Sand
- ☐ Mattress
- ☐ Rubber mat
- ☐ Other *(Please specify)*: _____

f) What type of **bedding** is used for your maternity pens? *(Check all that apply)*

- ☐ Not applicable
- ☐ Straw
- ☐ Sawdust
- ☐ Shavings
- ☐ Sand
- ☐ No bedding
- ☐ Other *(Please specify)*: _____

g) How often do you **clean out manure** from the maternity pens?

- ☐ Not applicable
- ☐ Never
- ☐ _____times/days
  (Number)
- ☐ Other *(Please specify)*: _____

h) How often do you **add new bedding** in the maternity pens?

- ☐ Not applicable
- ☐ Never
- ☐ _____times/day
  (Number)
- ☐ _____times/week
  (Number)

i) How often do you **remove and replace the bedding** completely in the maternity pens?

- ☐ Not applicable
- ☐ Never
- ☐ After every calving
- ☐ _____times/month
  (Number)
- ☐ Other *(Please specify)*: _____

j) If you empty your maternity pens completely, do you **wash or disinfect** them? *(Check all that apply)*

- ☐ Not applicable
- ☐ Yes, pressure washed or brushed.
- ☐ Yes, disinfected with _____ *(Name of the product)*
- ☐ Yes, other *(Please specify)*: _____
- ☐ No

2) Biosecurity

14) Do you **buy** adult animals (cows and first calf heifers)?

- ☐ Yes:
  - _____ *(Number)* adult cows bought in the last 12 months (milking or dry)
  - _____ *(Number)* first-calf heifers already milking bought in the last 12 months
- ☐ No

a) If you buy animals, what do you do **prior to moving the animal to your farm** to make sure that their udder is healthy? *(Check all that apply)*

- ☐ Not applicable
- ☐ I take a milk sample from each quarter for bacteriological analysis
- ☐ I take a pooled milk sample of the 4 quarters for bacteriological analysis
- ☐ I perform a CMT (California Mastitis Test)
- ☐ I ask the seller about the Somatic Cells Count of the animal
- ☐ I do not make any udder health verifications prior to moving the animal
- ☐ Other *(Please Specify)*: _____
  _____

b) If you buy animals, what do you do **after you have moved the animal to your farm** to make sure that their udder is healthy? *(Check all that apply)*

- ☐ Not applicable
- ☐ I take a milk sample from each quarter for bacteriological analysis
- ☐ I take a pooled milk sample of the 4 quarters for bacteriological analysis
- ☐ I perform a CMT (California Mastitis Test)
- ☐ I ask the seller about the Somatic Cells Count of the animal
- ☐ I do not make any udder health verifications after I have moved the animal
- ☐ Other *(Please Specify)*: _____
  _____

c) If an animal that you have bought is found to have an unhealthy udder based on the verification you have made, what do you do?

- ☐ Not applicable
- ☐ I return this animal to the seller
- ☐ I keep the animal
- ☐ Other *(Please Specify)*: _____

3) Diseases

15) Do you keep a **record of the diseases** occurring on your farm?

- ☐ Yes
- ☐ No

16) Do you have a vaccination program **against mastitis**?

- ☐ Yes, the vaccine(s) I use is (are) _____ *(List all)*
  (Vaccine's name)
- ☐ No

17) Do you have a **general vaccination program** for your adult cows?

- ☐ Yes, the vaccine(s) I use is (are) _____ *(List all)*
  (Vaccine's name)
- ☐ No

4) Cow management

18) Are your cows' udders clipped and/or flamed?

- ☐ Yes, clipped
- ☐ Yes, flamed
- ☐ No

19) Do you dock, clip or tie your cows' tails?

- ☐ Yes, docked
- ☐ Yes, clipped
- ☐ Yes, tied
- ☐ No

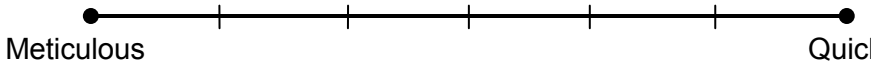
20) Do you use **electrical cow trainers**?

- ☐ Yes
- ☐ No

.

5) Personnal

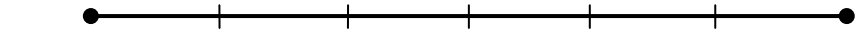
21) Place an X at the point of the scale which you think best describes your work habits.



Meticulous — Quick

22) Place an X at the point of the scale which you think best describes how you feel about milking.

☐ Not applicable (Not milking)



I enjoy milking — I prefer other tasks than milking

23) Place an X at the point of the scale which you think best describes your record keeping.

Minimal required quantity of information — Maximal quantity of information

24) How important is a persistent high somatic cell count in your culling decisions?
(Rank from 1 to 5, 1 being very important and 5 being not important)

Very important — Neutral — Not important

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

25) How important is an infection with Staphylococcus aureus in your culling decisions? *(Rank from 1 to 5, 1 being very important and 5 being not important)*

Very important — Neutral — Not important

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

26) At what level of somatic cell counts do you consider a cow a **high** somatic cell count cow?

At _____ *cells/ml*

27) At what **Bulk Milk Somatic Cell Count** level do you think you have a mastitis problem?

At _____ *cells/ml*

28) Do you agree with the following statements?

	Disagree		Neutral		Agree
a) High SCC cows are easy to discover during milking……....	1 ☐	2☐	3☐	4☐	5☐
b) I am concerned about the costs of cows with high SCC……	1 ☐	2☐	3☐	4☐	5☐
c) To prevent Staphylococcus aureus infections, it is more important to look at stall's cleanliness instead of milking procedures……	1 ☐	2☐	3☐	4☐	5☐
d) On my farm, udder health is an important aspect in bull selection……	1 ☐	2☐	3☐	4☐	5☐
e) Analysis of cows individual SCCs is very important…….....	1 ☐	2☐	3☐	4☐	5☐
f) Generally you cannot influence causes of sub-clinical mastitis……	1 ☐	2☐	3☐	4☐	5☐
g) I know enough about mastitis to keep me out of trouble…..	1 ☐	2☐	3☐	4☐	5☐
h) I should do more about mastitis prevention……..….	1☐	2☐	3☐	4☐	5☐

29) How often do you use these people to prevent or solve mastitis problems?

	Never		Sometimes		Regularly
a) Veterinarian……..……	1 ☐	2☐	3☐	4☐	5☐
b) DHI or Valacta representative…..…..	1 ☐	2☐	3☐	4☐	5☐
c) Nutritionist……..….	1 ☐	2☐	3☐	4☐	5☐
d) Milking equipment representative…..	1 ☐	2☐	3☐	4☐	5☐
e) Other dairy producers..……	1 ☐	2☐	3☐	4☐	5☐
f) Other: (Please specify)_____	1☐	2☐	3☐	4☐	5☐

Second part

6) Milking facilities:

30) Do you milk cows in a milking parlor or do you have to move the milking units from one cow to another?

- ☐ Milking parlor
- ☐ Milking units moved from cow to cow

31) What technique do you use to keep your cows standing after milking? *(Check all that apply)*

- ☐ I distribute fresh feed
- ☐ I keep cows locked in head-locks for _____ minutes. *(Duration)*
- ☐ I keep cows in a waiting area for _____ minutes. *(Duration)*
- ☐ I do not use a technique to keep cows standing
- ☐ Other *(Please specify)*: _____

32) Do you have milking units equipped with **automatic take-off**?

- ☐ Yes
- ☐ No

33) How many **milking units** do you currently use?

_____ Milking units
(Number)

7) Milking procedures

34) How often do you take part in milking cows?

_____% of milkings
(Number)

35) In the last 3 months, how many different people have milked cows on your farm?

_____ people
(Number)

36) If there are different people milking cows on your farm, do all of them use the same **milking routine**?

- ☐ Not applicable
- ☐ Yes
- ☐ No

37) If there are different people milking cows on your farm, how often do you **train** them?

- ☐ Not applicable
- ☐ Never
- ☐ Once, when they are hired
- ☐ _____ times/year
  (Number)
- ☐ Other *(Please specify)*: _____

38) Do your milkers get a bonus or other incentive if the milk reaches good quality targets?

- ☐ Not applicable
- ☐ Yes
- ☐ No

39) Do you have **written milking procedures** for your milkers?

- ☐ Yes
- ☐ No

40) How many **people** are milking cows at a typical milking?

_____ people
(Number)

41) Do the milkers wear **latex gloves** (or similar) during milking?

- ☐ Yes, all milkers
- ☐ Yes, some milkers
- ☐ No

a) If the milkers wear latex gloves, do they clean them regularly during milking?

- ☐ Not applicable
- ☐ Yes, rinse with water
- ☐ Yes, rinse in a disinfecting solution
- ☐ No
- ☐ Other *(Please specify)*: _____

42) Do you use a **pre-milking** teat dip?

- ☐ Yes
- ☐ No

a) If you use a **pre-milking** teat dip, how do you apply it?

☐ Not applicable
☐ Dipping
☐ Spraying
☐ Foaming
☐ Other *(Please specify)*: _____

b) If you use a **pre-milking** teat dip, what product do you use?

☐ Not applicable
☐ _____
(Name of the product)

c) If you use a **pre-milking** teat dip, how often do you clean the dispenser used to apply it?

☐ Not applicable
☐ Never
☐ _____times/day
(Number)

☐ _____times/month
(Number)

43) Do you ever **fore-strip** milk prior to attaching the milking unit?

☐ Yes
☐ No

a) If you fore-strip milk, in which situation(s) do you do it? *(Check all that apply)*

☐ Not applicable
☐ On every cow, at every milking
☐ On cows that are suspect for mastitis
☐ On cows with an elevated Somatic Cells Count (SCC)
☐ On cows that have clinical mastitis
☐ Other *(Please specify)*: _____

b) If you fore-strip milk, where do you discard the fore-stripped milk?

☐ Not applicable
☐ In a filter-cup
☐ On the floor underneath the cow
☐ In the milkers' hands
☐ Other *(Please specify)*: _____

44) Do you **clean teats** before installing the milking unit?

- ☐ Yes, I clean all teats
- ☐ Yes, I clean dirty teats only
- ☐ No, I do not clean teats

a) If you clean teats, how do you clean them?

- ☐ Not applicable
- ☐ Dry wipe
- ☐ Clean with pre-milking teat-dip
- ☐ Clean with water with udder wash
- ☐ Clean with water (without udder wash)
- ☐ Clean with commercially available wet disinfecting towel (e.g. .: Ready-wipe®…)
- ☐ Other *(Please specify)*: _____

45) How do you **dry teats** prior to attaching the milking unit?

- ☐ Not applicable
- ☐ Disposable paper towel (or newspaper)
- ☐ Reusable cloth towel
- ☐ I do not dry teats
- ☐ Other *(Please specify)*: _____

a) If you do **dry teats**, do you use the same towel to dry teats of different cows during the same milking?

- ☐ Not applicable
- ☐ Yes
- ☐ No

b) If you use **reusable towel**, do you wash or disinfect these towels after every milking?

- ☐ Not applicable
- ☐ Yes
- ☐ No

46) Do you use a **post-milking** teat-dip?

- ☐ Yes
- ☐ No

a) If you use a **post-milking** teat-dip, how do you apply it?

☐ Not applicable
☐ Dipping
☐ Spraying
☐ Other *(Please specify)*: _____

b) If you use a **post-milking** teat-dip, what product do you use?

☐ Not applicable
☐ _____
(Name of the product)

c) If you use a **post-milking** teat-dip, how often do you clean the dispenser used to apply it?

☐ Not applicable
☐ Never
☐ _____times/day
(Number)

☐ _____times/month
(Number)

47) Do you clean the milking units between each cow during milking?

☐ Yes, a backflush is done between each cow
☐ Yes, the milking units are dipped in a disinfecting solution between each cow
☐ No
☐ Other*(Please specify)*:_____

8) Milking equipment

48) How often do you have your milking equipment **inspected** by a certified technician?

☐ Never
☐ _____times/year
(Number)

49) How often do you verify the vacuum level?

☐ Never
☐ _____times/week
(Number)

☐ _____times/month
(Number)

☐ _____times/year
(Number)

50) Do you replace the **teat cup liners** at the frequency recommended by the dealer?

☐ Yes
☐ No, I keep them longer
☐ No, I change them more often

9) Clinical mastitis:

51) Do you measure **milk conductivity** (conductimeter) in order to detect mastitis?

☐ Yes
☐ No

52) On your farm, what proportion of cows with clinical mastitis are treated with antibiotics?

_____%
(Number)

53) How important are these factors when deciding whether to treat a cow with mastitis with antibiotics? *(Rank from 1 to 5, 1 being a very important factor and 5 being a factor that is not important)*

	Very important		Neutral		Not important
a) Production, age, and genetics of the cow……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
b) Severity of the symptoms……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
c) Need for milk to fill quota……..	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
d) Cull cow price and price to buy a new milking cow… .	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
e) Protocol established with my veterinarian……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
f) Other *(Please specify)*:_____..	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

54) When you treat a cow for mastitis, what is the average **duration** of your treatment?

- ☐ Not applicable
- ☐ _____ Days
  (Number)

55) Which **product** do you use as a first treatment for mastitis?

- ☐ Not applicable
- ☐ Special Formula 17900 Forte®
- ☐ Cefa-Lak®
- ☐ Pirsue®
- ☐ Erythro-36®
- ☐ Dolisovet®
- ☐ Other *(Please specify)*: _____

56) When you use an intramammary treatment, in what proportion of treatments do you **disinfect the teat end** with an alcohol swab prior to administration?

- ☐ Not applicable
- ☐ _____%
  (Number)

57) When you use an intramammary treatment, do you use a partial insertion (short tip) or a complete insertion (long tip)?

- ☐ Not applicable
- ☐ Partial insertion
- ☐ Complete insertion

58) When you use an intramammary treatment, in what proportion of treatments do you apply a **teat dip** after the administration?

- ☐ Not applicable
- ☐ _____%
  (Number)

10) Subclinical mastitis

59) Do you monitor **individual cow production** at every milking?

- ☐ Yes
- ☐ No

60) How often do you review the individual **Somatic Cells Count** (SCC) data of your cows?

- ☐ Every month, the day I receive my DHI report.
- ☐ Every month, as soon as I have time to do it.
- ☐ When I have mastitis problems
- ☐ Never
- ☐ Other *(Please specify)*:_____

61) Do you use the CMT test (**California Mastitis Test**) on a regular basis in order to detect subclinical mastitis?

- ☐ Yes
- ☐ No

62) Do you take milk samples (for bacteriological analysis) regularly in order to detect cows that are suffering from **subclinical mastitis**?

- ☐ Yes, most of my cows are sampled once a year
- ☐ Yes, suspect cows are sampled
- ☐ No

63) Do you milk cows that have **chronic infection** (ex: *Staph aureus*) last **or** with a specific milking unit?

- ☐ Yes, last
- ☐ Yes, with a specific milking unit
- ☐ No
- ☐ Other *(Please specify)*:_____

Annexe II. Questionnaire santé du pis (Français)

Première partie:

1) Logement:

Vaches en lait

1) Type de logement pour les vaches en **lactation**:

☐ Stabulation entravée
☐ Stabulation libre à logettes
☐ Stabulation libre sur litière accumulée
☐ Autre *(SVP précisez)*: _____
_____

a) Si vous avez une **stabulation libre**, de quelle manière les **allées** sont-elles nettoyées?

☐ Ne s'applique pas
☐ Planché latté
☐ Grattées
☐ Rincées à l'eau
☐ Autre *(SVP précisez)*: _____

b) Si vous avez une **stabulation libre**, combien de fois par jour les allées sont-elles nettoyées?

☐ Ne s'applique pas
☐ _____ fois/jour.
(Nombre)

2) Est-ce que vos **vaches en lactation** ont eu accès au pâturage dans les **derniers 12 mois**?

☐ Non, elles sont gardées à l'intérieur à l'année longue.
☐ Non, mais elles ont eu accès à une cours d'exercice gazonnée (moins de 5 acres par 100 vaches)
☐ Non, mais elles ont eu accès à une cours d'exercice non-gazonnée (Pavée ou terre) (moins de 5 acres par 100 vaches)
☐ Oui, elles étaient au pâturage à partir du mois de _____ jusqu'au mois de _____.
(Mois) *(Mois)*

3) De quel type de matériel est constitué **la base** des logettes (ou des parcs pour les étables sur litière accumulée) de vos **vaches en lactation**? *(Cochez tout ce qui s'applique)*

- ☐ Ciment
- ☐ Sable
- ☐ Matelas (ex: Pasture mat...)
- ☐ Tapis de caoutchouc
- ☐ Autre *(SVP précisez)*: _____

4) Quel type de **litière** est utilisée pour les logettes (ou les parcs pour les étables sur litière accumulée) de vos **vaches en lactation**? *(Cochez tout ce qui s'applique)*

- ☐ Paille
- ☐ Sciure de bois
- ☐ Copeaux de bois
- ☐ Sable
- ☐ Aucune litière
- ☐ Autre *(SVP précisez)*: _____

5) Quel genre de **régie de la litière** utilisez-vous?

- ☐ Je n'utilise pas de litière
- ☐ J'utilise une très petite quantité de litière (**moins de 2 cm d'épais**)
- ☐ J'utilise une litière épaisse (**plus de 2 cm d'épais**)
- ☐ Autre *(SVP préciser)*: _____

6) À quelle fréquence **retirez-vous les fumiers** dans les logettes (ou les parcs pour les étables sur litière accumulée) de vos **vaches en lactation**?

- ☐ Jamais
- ☐ _____fois/jour
  (Nombre)
- ☐ Autre *(SVP préciser)*: _____

7) À quelle fréquence **grattez-vous la litière sale** hors des logettes (ou les parcs pour les étables sur litière accumulée) de vos **vaches en lactation**?

- ☐ Ne s'applique pas
- ☐ Jamais
- ☐ _____fois/jour
  (Nombre)
- ☐ _____fois/semaine
  (Nombre)

a) Si vous **grattez la litière sale** hors des logettes de vos **vaches en lactation** quelle partie de la logette grattez-vous complétement?

- ☐ Ne s'applique pas
- ☐ Le tier arrière de la logette
- ☐ La moitié arrière de la logette
- ☐ La logette entière
- ☐ Autre *(SVP préciser)*: _____

8) À quelle fréquence **ajoutez-vous de la nouvelle litière** dans les logettes (ou les parcs pour les étables sur litière accumulée) de vos **vaches en lactation**?

- ☐ Ne s'applique pas

- ☐ _____fois/jour
  (Nombre)
- ☐ _____fois/semaine
  (Nombre)
- ☐ _____fois/an
  (Nombre)

9) À quelle fréquence **retirez-vous et remplacez-vous complètement** la litière des logettes (ou les parcs pour les étables sur litière accumulée) de vos **vaches en lactation**?

- ☐ Ne s'applique pas

- ☐ Jamais
- ☐ _____fois/semaine
  (Nombre)
- ☐ _____fois/mois
  (Nombre)
- ☐ Autre *(SVP précisez)*: _____

10) Quand vous videz les logettes complètement, sont-elles **lavées** ou **désinfectées**? *(Cochez tout ce qui s'applique)*

- ☐ Ne s'applique pas
- ☐ Oui, lavées à la pression ou brossées
- ☐ Oui, désinfectées avec _____
  (Nom du produit)
- ☐ Oui, autre *(SVP précisez)*: _____
- ☐ Non

11) Ajoutez-vous un produit afin de limiter la croissance bactérienne dans la litière et/ou dans les logettes de vos **vaches en lactation**? (ex: chaux...)

- ☐ Oui *(SVP précisez le produit utilisé et la fréquence d'utilisation)*:_____
  _____
- ☐ Non

12) Utilisez-vous une méthode de contrôle des mouches pour la saison estivale? (ex : insecticide, pièges…)

☐ Oui
☐ Non

13) Possédez-vous un parc de vêlage?

☐ Oui, j'ai _____ parcs de vêlage
(Nombre)
☐ Non

a) Utilisez-vous les parcs de vêlage pour d'autres usages que le vêlage (ex: vaches malades, vaches boîteuses, ...)?

☐ Ne s'applique pas
☐ Oui
☐ Non

b) Quel **pourcentage de vos vêlages** se produit dans un parc de vêlage?

☐ Ne s'applique pas
☐ _____% des vêlages

c) Combien de **jours** vos vaches demeurent-elles dans le parc de vêlage?

☐ Ne s'applique pas
☐ _____ Jours avant le vêlage et _____ jours après le vêlage
(Nombre) *(Nombre)*

d) Gardez-vous plus d'une vache par parc de vêlage?

☐ Ne s'applique pas
☐ Non
☐ Oui

e) De quel type de matériel est constitué la **base de vos parcs de vêlage**?
(Cochez tout ce qui s'applique)

☐ Ne s'applique pas

☐ Ciment
☐ Sable
☐ Matelas
☐ Tapis de caoutchouc
☐ Autre *(SVP précisez)*: _____

f) Quel type de **litière** utilisez-vous dans les parcs de vêlage? *(Cochez tout ce qui s'applique)*

☐ Ne s'applique pas

☐ Paille
☐ Sciure de bois
☐ Copeaux de bois
☐ Sable
☐ Aucune litière
☐ Autre (précisez): _____

g) À quelle fréquence ramassez-vous les fumiers dans les parcs de vêlage?

☐ Ne s'applique pas
☐ Jamais

☐ _____ fois/jour
(Nombre)

☐ Autre *(SVP précisez)*: _____

h) À quelle fréquence **ajoutez-vous de la nouvelle litière** dans les parcs de vêlage?

☐ Ne s'applique pas
☐ Jamais

☐ _____ fois/jour
(Nombre)

☐ _____ fois/semaine
(Nombre)

i) À quel fréquence **retirez-vous et remplacez-vous complétement la litière** des parcs de vêlage?

☐ Ne s'applique pas
☐ Jamais
☐ Après chaque vêlage

☐ _____ fois/mois
(Nombre)

☐ Autre *(SVP précisez)*: _____

k) Si vous videz vos parcs de vêlage complètement, sont-ils **lavés ou désinfectés**? *(Cochez tout ce qui s'applique)*

- ☐ Ne s'applique pas
- ☐ Oui, lavés à la pression ou brossés.
- ☐ Oui, désinfectés avec _____ *(Nom du produit)*
- ☐ Oui, autre *(SVP précisez)*: _____
- ☐ Non

2) Biosécurité

14) Est-ce que vous **achetez** des animaux adultes (vaches et taures premier veau)?

- ☐ Oui: _____ *(Nombre)* vaches adultes achetées dans les derniers 12 mois (en lait ou taries)
  _____ *(Nombre)* taures premier veau déjà en lactation achetées dans les derniers 12 mois
- ☐ Non

a) Si vous achetez des animaux, que faite-vous **avant d'ammener l'animal à votre ferme** pour vous assurez que leur pis est sain? *(Cochez tout ce qui s'applique)*

- ☐ Ne s'applique pas
- ☐ Je prends un échantillon de lait de chaque quartier pour analyse bactériologique
- ☐ Je prends un échantillon composite de lait des 4 quartiers pour analyse bactériologique
- ☐ Je fais un test CMT (California Mastitis Test)
- ☐ Je m'informe auprès du vendeur sur le comptage des cellules somatiques (CCS) de l'animal.
- ☐ Je ne fais pas de vérifications de la santé du pis avant d'ammener l'animal
- ☐ Autre *(SVP précisez)*:_____
- ☐ _____

b) Si vous achetez des animaux, que faite-vous **après avoir ammené l'animal à votre ferme** pour vous assurez que leur pis est sain? *(Cochez tout ce qui s'applique)*

- ☐ Ne s'applique pas
- ☐ Je prends un échantillon de lait de chaque quartier pour analyse bactériologique
- ☐ Je prends un échantillon composite de lait des 4 quartiers pour analyse bactériologique
- ☐ Je fais un test CMT (California Mastitis Test)
- ☐ Je m'informe auprès du vendeur sur le comptage des cellules somatiques (CCS) de l'animal.
- ☐ Je ne fais pas de vérifications de la santé du pis après avoir ammené l'animal
- ☐ Autre *(SVP précisez)*:_____
  _____

c) Si un animal que vous avez acheté a un pis qui n'est pas sain, basé sur la vérification que vous avez faite, que faites-vous?

- ☐ Ne s'applique pas
- ☐ Je retourne cet animal au vendeur
- ☐ Je garde cet animal
- ☐ Autre *(SVP précisez)*: _____

3) Maladies

15) Est-ce que vous gardez un **dossier des maladies** qui surviennent sur votre ferme?

- ☐ Oui
- ☐ Non

16) Avez-vous un programme de vaccination **contre la mammite**?

- ☐ Oui, le(s) vaccin(s) que j'utilise est (sont) _____
  (Nom du (des) vaccin(s))
- ☐ Non

17) Avez-vous un programme de vaccination générale?

- ☐ Oui, le(s) vaccin(s) que j'utilise est (sont) _____
  (Nom du (des) vaccin(s))
- ☐ Non

4) Régie des vaches

18) Est-ce que les pis de vos vaches sont rasés et/ou flambés?

- ☐ Oui, rasés
- ☐ Oui, flambés
- ☐ Non

19) Est-ce que vous attachez ou coupez la queue ou les poils de la queue de vos vaches?

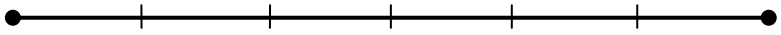
- ☐ Oui, les queues sont coupées
- ☐ Oui, les poils de la queue sont coupés
- ☐ Oui les queues sont attachées
- ☐ Non

20) Utilisez-vous des **dresseurs électriques**?

- ☐ Oui
- ☐ Non

5) Personnel

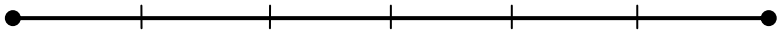
21) Placez un X sur l'endroit de l'échelle qui, selon vous, décrit le mieux **vos habitudes de travail**?



Méticuleux — Rapide

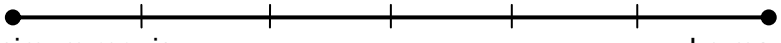
22) Placez un X sur l'endroit de l'échelle qui, selon vous, décrit le mieux **comment vous vous sentez à propos de la traite**?

☐ Ne s'applique pas (ne tire pas les vaches)



J'aime tirer les vaches — Je préfère d'autre tâches que la traite

23) Placez un X sur l'endroit de l'échelle qui, selon vous, décrit le mieux **votre tenue de dossier**?



Le minimum requis d'informations — Le maximum d'informations

24) Quelle est l'importance d'un comptage des cellules somatiques élevé de manière persistente dans vos décisions de réforme? *(Classer de 1 à 5, 1 étant très important et 5 pas important)*

Très important		Neutre		Pas important
1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

25) Quelle est l'importance d'une infection à Staphylococcus aureus dans vos décisions de réforme? *(Classer de 1 à 5, 1 étant très important et 5 pas important)*

Très important		Neutre		Pas important
1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

26) À partir de quel niveau de comptage des cellules somatiques considérez-vous qu'une vache a un comptage des cellules somatiques élevé?

À_____ cellules/ml

27) À partir de quel niveau de **comptage des cellules somatiques du réservoir de lait** pensez-vous avoir un problème de mammite?

À_____ cellules/ml

28) Êtes-vous en encore avec les énoncés suivants?

	En désaccord		Neutre		En accord
a) Les vaches à haut CCS sont faciles à détecter lors de la traite……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
b) Je suis préoccupé par les coûts dus aux vaches avec un CCS élevé……..	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
c) Afin de prévenir les infections à Staphylococcus aureus, il est plus important de surveiller la propreté des logettes plutôt que la technique de traite…….	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
d) Sur ma ferme, la santé du pis est un aspect important lors de la sélection des taureaux…….……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
e) L'analyse du CCS individuels des vaches est très importante……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
f) En général, on ne peut pas influencer les causes de mammites sub-cliniques……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

g) J'en connais assez sur la mammite pour éviter les problèmes..... 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

h) Je devrais en faire plus à propos de la prévention de la mammite..... 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

29) À quelle fréquence faites-vous appel à ces personnes ressources afin de prévenir ou résoudre des problèmes de mammite?

	Jamais		Parfois		Régulièrement
a) Vétérinaire……..……....	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
b) Représentant Valacta……..…..…..	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
c) Nutritionniste……..……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
d) Représentant d'équipement de traite.	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
e) D'autres producteurs laitiers……	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
f) Autre: (SVP précisez)_____	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

Deuxième partie

6) Installations de traite

30) Est-ce que vos vaches sont traites dans un salon de traite ou devez-vous déplacez les trayeuses d'une vache à l'autre?

☐ Salon de traite
☐ Unités de traite déplacées d'une vache à l'autre

31) Quelle technique utilisez-vous pour garder vos vaches debout après la traite? *(Cochez tout ce qui s'applique)*

☐ Je distribue des aliments frais.
☐ Je verrouille les portes cornadis pour _____ minutes. *(Durée)*
☐ Je laisse les vaches dans une aire d'attente pour _____ minutes. *(Durée)*
☐ Je n'utilise pas de technique pour garder les vaches debout
☐ Autre *(SVP précisez)*: _____

32) Est-ce que vous avez des trayeuses équipées de **retraits-automatiques**?

- ☐ Oui
- ☐ Non

33) Combien **d'unités de traite** utilisez-vous actuellement?

_____ Unités de traite
(Nombre)

7) Procédures de traite

34) À quelle fréquence prenez-vous part à la traite des vaches?

_____ % des traites
(Nombre)

35) Dans les derniers 3 mois, combien de personne différentes ont tiré les vaches sur votre ferme?

_____ personnes
(Nombre)

36) Si il y a différentes personnes qui traient les vaches sur votre ferme, est-ce qu'elles utilisent tous la même **routine de traite**?

- ☐ Ne s'applique pas
- ☐ Oui
- ☐ Non

37) Si il y a différentes personnes qui traient les vaches sur votre ferme, à quelle fréquence les **formez**-vous?

- ☐ Ne s'applique pas
- ☐ Jamais
- ☐ Une seul fois, lors de leur engagement
- ☐ _____ fois/année
  (Nombre)
- ☐ Autre *(SVP précisez)*: _____

38) Est-ce que vos trayeurs reçoivent un bonus ou d'autre incentif si le lait atteint des standards de bonne qualité?

- ☐ Ne s'applique pas
- ☐ Oui
- ☐ Non

39)Avez-vous une **méthode de traite écrite** pour vos trayeurs?

☐ Oui
☐ Non

40)Combien de **personnes** traient les vaches lors d'une traite typique?

_____ personnes
(Nombre)

41)Est-ce que les trayeurs portent des **gants de latex** (ou similaire) durant la traite?

☐ Oui, tous
☐ Oui, quelques-uns
☐ Non

b) If the milkers wear latex gloves, do they clean them regularly during milking?

☐ Not applicable
☐ Yes, rinse with water
☐ Yes, rinse in a disinfecting solution
☐ No
☐ Other *(Please specify)*: _____

42)Utilisez-vous un bain de trayon **pré-traite**?

☐ Oui
☐ Non

a) Si vous utilisez le bain de trayon **pré-traite**, de quel manière l'appliquez-vous?

☐ Ne s'applique pas
☐ Trempage
☐ Pulvérisation
☐ Mousse
☐ Autre *(SVP précisez)* :_____

b) Si vous utilisez le bain de trayon pré-traite, quel produit(s) utilisez-vous?

☐ Ne s'applique pas
☐ _____
(Nom du produit)

c) Si vous utilisez le bain de trayon **pré-traite**, à quelle fréquence nettoyez-vous le contenant servant à l'application?

☐ Ne s'applique pas
☐ Jamais
☐ _____ fois/jour
(Nombre)

☐ _____ fois/mois
(Nombre)

43)Retirez-vous parfois les **premier jets** de lait avant d'attacher l'unité de traite?

☐ Oui
☐ Non

a) Si vous tirez les premier jets de lait, dans quel(s) cas le faites-vous?
(Cochez tout ce qui s'applique)

☐ Ne s'applique pas
☐ Sur toutes les vaches, à chaque traite
☐ Sur les vaches qui sont suspecte pour la mammite
☐ Sur les vaches avec un Comptage des Cellules Somatiques (CCS) élevé
☐ Sur les vaches avec une mammite clinique
☐ Autre *(SVP précisez)*:_____

b) Si vous tirez les premier jets, où jetez-vous les premiers jets de lait?

☐ Ne s'applique pas
☐ Dans une tasse-filtre
☐ Sur le plancher sous la vache
☐ Sur les mains des trayeurs
☐ Autre *(SVP précisez)*: _____

44)Nettoyez-vous les trayons avant d'installer l'unité de traite?

☐ Oui, je nettoie tout les trayons
☐ Oui, je nettoie les trayons sales seulement
☐ Non, je ne nettoie pas les trayons

a) Si vous nettoyez les trayons, de quelle manière les nettoyez-vous?

- ☐ Ne s'applique pas
- ☐ Essuyage à sec
- ☐ Nettoyage avec le bain de trayon pré-traite
- ☐ Nettoyage avec de l'eau contenant une solution lave-pis
- ☐ Nettoyage avec de l'eau (sans solution lave-pis)
- ☐ Nettoyage avec des serviettes humides désinfectantes disponibles commercialement (ex: Ready-Wipe®...)
- ☐ Autre *(SVP précisez)* :_____

45)De quelle manière **séchez-vous les trayons** avant d'attacher l'unité de traite?

- ☐ Ne s'applique pas
- ☐ Serviettes en papier disposables (ou papier journal)
- ☐ Serviettes en tissus réutilisables
- ☐ Je ne sèche pas les trayons
- ☐ Autre *(SVP précisez)*:_____

a) Si vous **séchez les trayons**, utilisez-vous la même serviette pour sécher les trayons de différentes vaches lors d'une même traite?

- ☐ Ne s'applique pas
- ☐ Oui
- ☐ Non

b) Si vous utilisez des **serviettes réutilisables**, lavez-vous ou désinfectez-vous ces serviettes après chaque traite?

- ☐ Ne s'applique pas
- ☐ Oui
- ☐ Non

46)Utilisez-vous un bain de trayon **post-traite**?

- ☐ Oui
- ☐ Non

a) Si vous utilisez le bain de trayon **post-traite**, de quel manière l'appliquez-vous?

- ☐ Ne s'applique pas
- ☐ Trempage
- ☐ Pulvérisation
- ☐ Autre *(SVP précisez)*: _____

b) Si vous utilisez le bain de trayon **post-traite**, quel produit(s) utilisez-vous?

☐ Ne s'applique pas
☐ _____
(Nom du produit)

c) Si vous utilisez le bain de trayon **post-traite**, à quelle fréquence nettoyez-vous le contenant servant à son application?

☐ Ne s'applique pas
☐ Jamais
☐ _____ fois/jour
(Nombre)

☐ _____ fois/mois
(Nombre)

47) Nettoyez-vous les unités de traite entre chaque vache durant la traite?

☐ Oui, un rinçage à circulation inversée (*backflush*) est effectué entre chaque vache.
☐ Oui, les unités de traite sont trempées dans une solution désinfectante entre chaque vache.
☐ Non
☐ Autre *(SVP précisez)*:_____

8) Équipement de traite

48) À quelle fréquence faites-vous **inspecter** votre équipement de traite par un technicien certifié?

☐ Jamais
☐ _____ fois/an
(Nombre)

49) À quelle fréquence vérifiez-vous le niveau de vide?

☐ Jamais
☐ _____ fois/semaine
(Nombre)

☐ _____ fois/mois
(Nombre)

☐ _____ fois/an
(Nombre)

50) Remplacez-vous les **gobelets-trayeurs** à la fréquence recommandée par le distributeur?

☐ Oui
☐ Non, je les garde plus longtemps.
☐ Non, je les change plus fréquemment.

9) Mammite clinique

51) Mesurez-vous la **conductivité du lait** (conductimètre) afin de détecter la mammite?

☐ Oui
☐ Non

52) Sur votre ferme, quelle proportion des vaches avec une mammite clinique sont traitées avec des antibiotiques?

_____%
(Nombre)

53) Quelle est l'importance de ces facteurs lorsque vous décidez de traiter ou non une vache atteinte de mammite avec des antibiotiques? *(Classer de 1 à 5, 1 étant un facteur très important et 5 étant un facteur qui n'est pas important)*

	Très important		Neutre		Pas important
a) La production, l'âge et la génétique de la vache ……..1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	
b) La sévérité des symptômes ……... 1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	
c) Le besoin de lait pour remplir le quota …….. 1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	
d) Le prix des vaches de réforme et le prix pour acheter une nouvelle vache laitière……..…….. 1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	
e) Le protocole établi avec mon vétérinaire …… 1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	
f) Autre *(SVP précisez)* :_____ 1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	

54) Lorsque vous traitez une vache pour mammite, quelle est la **durée** moyenne de votre traitement?

☐ Ne s'applique pas
☐ _____ Jours
(Nombre)

55) Quel **produit** utilisez-vous comme premier traitement pour la mammite?

- ☐ Ne s'applique pas
- ☐ Special Formula 17900 Forte®
- ☐ Cefa-Lak®
- ☐ Pirsue®
- ☐ Erythro-36®
- ☐ Dolisovet®
- ☐ Autre *(SVP précisez)*: _____

56) Lorsque vous utilisez un traitement intramammaire, dans quelle proportion des traitements **désinfectez-vous le bout du trayon** avec un tampon d'alcool avant l'administration?

- ☐ Ne s'applique pas
- ☐ _____% *(Nombre)*

57) Lorsque vous utilisez un traitement intramammaire, utilisez-vous une insertion partielle (embout court) ou une insertion complète (embout long)?

- ☐ Ne s'applique pas
- ☐ Insertion partielle
- ☐ Insertion complète

58) Lorsque vous utilisez un traitement intramammaire, dans quelle proportion des traitements appliquez-vous un **bain de trayon** après l'administration?

- ☐ Ne s'applique pas
- ☐ _____% *(Nombre)*

10) Mammite subclinique

59) Surveillez-vous la **production individuelle de vos vaches** à chaque traite?

- ☐ Oui
- ☐ Non

60) À quelle fréquence vérifiez-vous les données de **Comptage des Cellules Somatiques** (CCS) individuelles de vos vaches?

- ☐ À tout les mois, le jour même que je reçois mon rapport Valacta.
- ☐ À tout les mois, aussitôt que j'ai le temps de le faire.
- ☐ Quand j'ai des problèmes de mammite.
- ☐ Jamais
- ☐ Autre *(SVP précisez)*: _____

61) Utilisez-vous le test CMT (**California Mastitis Test**) de manière régulière afin de détecter la mammite sub-clinique?

- ☐ Oui
- ☐ Non

62) Prenez-vous des échantillons de lait (pour analyse bactériologique) de manière régulière afin de détecter les vaches souffrant de **mammite subclinique**?

- ☐ Oui, la plupart de mes vaches sont échantillonnées une fois par année
- ☐ Oui, les vaches suspectes sont échantillonnées
- ☐ Non

63) Trayez-vous les vaches qui ont une **mammite chronique** (ex : *Staph. aureus*) en dernier **ou** avec une unité de traite spécifique?

- ☐ Oui, en dernier
- ☐ Oui, avec une unité de traite spécifique
- ☐ Non
- ☐ Autre *(SVP précisez)*:_____

Annexe III. Code WinBUGS utilisé pour modéliser l'erreur de mesure dans les analyses sur SCN

Modèle pour incidence et élimination d'IIM:

```
#-----------------------------------------------------#
# WinBugs model for CNS incidence data #
# Model 1: Response misclassification       #
#                                                     #
# Author: Simon Dufour                      #
# Created: 31 August 2011                   #
# last modified: Oct 2nd 2011               #
#-----------------------------------------------------#
#----MODEL Definition----------------
model
{
# Level 1 definition
for(i in 1:N) {
        IMI[i] ~ dbern(pstar[i])                    # IMI = 1 when CNS present,
                                                     #0 otherwise

        pstar[i]<-p[i]*Sey + (1-p[i])*(1-Spy)
        logit(p[i]) <- beta[1] + beta[2]*x[i] + .... #The usual logistic model should
                                                     # appears here with the betas.
                                                     # Betas have to be inverted if
                                                     # IMI elimination is predicted

                }
# Higher level definitions
for (j in 1:n2) {
u2[j] ~ dnorm(0,tau.u2)
}
for (j in 1:n3) {
u3[j] ~ dnorm(0,tau.u3)
}
for (j in 1:n4) {
u4[j] ~ dnorm(0,tau.u4)
}
# Priors for response Se and Sp:
Sey ~ dbeta(165,39)I(0.76, 0.86)
Spy ~ dbeta(145, 22.5)I(0.82, 0.92)
# Priors for fixed effects
for (k in 1:22) { beta[k] ~ dflat() }
# Priors for random terms
```

```
tau.u2 ~ dgamma(0.001000,0.001000)
sigma2.u2 <- 1/tau.u2
tau.u3 ~ dgamma(0.001000,0.001000)
sigma2.u3 <- 1/tau.u3
tau.u4 ~ dgamma(0.001000,0.001000)
sigma2.u4 <- 1/tau.u4
}
```

Modèle pour prévalence d'IIM:

```
#-----------------------------------------------------#
# WinBugs model for CNS prevalence data  #
# Model 1: Response misclassification         #
#                                                     #
# Author: Simon Dufour                        #
# Created: 31 August 2011                     #
# last modified: Sept 24th 2011               #
#-----------------------------------------------------#

#----MODEL Definition----------------
model
{
# Level 1 definition
for(i in 1:N) {
                IMI[i] ~ dbern(pstar[i])                    # IMI = 1 when CNS present,
                                                            #0 otherwise

                pstar[i]<-p[i]*Sey[samples[i]] + (1-p[i])*(1-Spy[samples[i]])

                                                            #"samples" is a variable for the
                                                            #number of
                                                            #culture results (1, 2, or 3) used
                                                            #to identify prevalence of an
                                                            #IMI

                logit(p[i]) <- beta[1] + beta[2]*x[i] + .... #The usual logistic model should
                                                            # appears here with the betas.

+ u2[cow_uni[i]] * Cons[i]
+ u3[herdprid[i]] * Cons[i]
+ u4[period[i]] * Cons[i]
}
# Higher level definitions
for (j in 1:n2) {
u2[j] ~ dnorm(0,tau.u2)
}
for (j in 1:n3) {
u3[j] ~ dnorm(0,tau.u3)
}
for (j in 1:n4) {
u4[j] ~ dnorm(0,tau.u4)
}
```

```
# Priors for response Se and Sp:
Sey[1] ~ dbeta(165,39)I(0.76, 0.86)
Spy[1]~ dbeta(145, 22.5)I(0.82, 0.92)

Sey[2] ~ dbeta(92,4.0)I(0.91,)
Spy[2]~ dbeta(174, 55.5)I(0.71,0.81)

Sey[3] ~ dbeta(68,1.5)I(0.93,)
Spy[3]~ dbeta(172, 89.0)I(0.61, 0.71)

# Priors for fixed effects
for (k in 1:34) { beta[k] ~ dflat() }
# Priors for random terms
tau.u2 ~ dgamma(0.001000,0.001000)
sigma2.u2 <- 1/tau.u2
tau.u3 ~ dgamma(0.001000,0.001000)
sigma2.u3 <- 1/tau.u3
tau.u4 ~ dgamma(0.001000,0.001000)
sigma2.u4 <- 1/tau.u4
}
```